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FINAL REPORT

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Principal Investigator

CARDIOVASCULAR SYSTEM

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13. ABSTRACT

The following significant findings were made during the past year. The ability of the heart to adapt to stress requires an intact autonomic nervous system. Acute increases in arterial pressure may cause detrimental effects to the system by direct action on the heart, particularly if underlying myocardial disease is present. The ability of the heart to adapt to different heart rates appears to be an important factor in exercise or prolonged hypoxia. Relaxing systems are important in the action of antihypertensive drugs and perhaps in the etiology and maintenance of abnormal blood pressure states. Reserpine mediated electrolyte loss from vascular tissue is the result of urinary excretion of sodium, potassium and calcium and calcium excretion into the gut. A new type of supersensitivity was discovered and characterized in vascular smooth muscle initiated by cold temperature. Prostaglandins augment myocardial contractility by increasing intracellular calcium stores. Cerebellar inhibitory mechanisms, but not reticular or spinal inhibitory mechanisms, were markedly suppressed by hallucinogenic drugs. Bicuculline suppressed cerebellar inhibition, but also suppressed reticular and presynaptic inhibition. There is a marked difference in the degree of inhibition and the rate of recovery from the inhibition in various tissues of the rat due to the insecticide disulfoton. The use of microwave radiation to rapidly inactive brain enzymes has been found to be a remarkably useful technique in the study of central neurotransmitters. Acetylcholinesterase increases in the hippocampal formation of the rat brain during shock avoidance learning.

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FINAL SUMMARY FOR AEROSPACE CONTRACT

The following are the significant scientific findings in the past
year for Aerospace Grant #AF 70-C-0059.

CARDIOVASCULAR:

1. The ability of the heart to adapt to stress requires an intact
    autonomic nervous system.

2. Acute increases in arterial pressure may cause detrimental
    effects to the system by direct action on the heart, particularly
    if underlying myocardial disease is present.

3. A pressure dimension instrument for assessing cardiac function
    has been developed and should be useful in detecting early
    dysfunctions on the heart.

4. The ability of the heart to adapt to different heart rates appears
    to be an important factor in exercise or prolonged hypoxia.

5. Relaxing systems are important in the action of antihypertensive
    drugs and perhaps in the etiology and maintenance of abnormal
    blood pressure states.

6. The release of calcium by electrical stimulation from cardiac
    sarcoplasmic reticulum is involved in force-frequency relations,
    paired stimulation and the action of pentobarbital.

7. Reserpine mediated electrolyte loss from vascular tissue is the
    result of urinary excretion of sodium, potassium and calcium and
    calcium excretion into the gut.

8. A new type of supersensitivity was discovered and characterized in
    vascular smooth muscle initiated by cold temperature.

9. The prostaglandins PGE_1 and $PGF_{2\alpha}$ augment myocardial contractility by increasing intracellular calcium stores.

CENTRAL NERVOUS SYSTEM:

1. Cerebellar inhibitory mechanisms, but not reticular or spinal inhibitory mechanisms, were markedly suppressed by hallucinogenic drugs. These observations are in support of the proposition that these drugs act upon the cerebellum to produce their abnormal motor effects.

2. Bicuculline, a convulsant GABA antagonist, similarly suppressed cerebellar inhibition, but also suppressed reticular and pre-synaptic inhibition. These observations support the proposition that GABA is involved in cerebellar and presynaptic inhibition, and also suggests that the suppression of cerebellar inhibition by the hallucinogenic drugs is not the result of any GABA blockade by these drugs.

3. There is a marked difference in the degree of inhibition and the rate of recovery from the inhibition in various tissues of the rat due to the insecticide disulfoton.

4. The use of microwave radiation to rapidly inactive brain enzymes has been found to be a remarkably useful technique in the study of central neurotransmitters.

5. The enzyme acetylcholinesterase increases in the hippocampal formation of the rat brain during shock avoidance learning.

6. Contrary to recent reports, acute or chronic ethanol administration to rats was found to have no significant impact upon the true or pseudocholinesterase activity in five areas of the brain.

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plasmic reticulum. (To be published).

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CARDIOVASCULAR SYSTEM

DETAILED PROGRESS REPORT

I. The Action of Hydralazine on Isolated Rabbit Aortic Strips

A. Progress. We have continued to study the effects of hydralazine, an antihypertensive compound, on calcium fluxes in isolated rabbit aortic strips.

B. Methods. Spiral strips of aorta were prepared and suspended in a muscle warmer containing oxygenated Ringer-Locke solution at 37°C. Tension was measured by a strain gauge Grass FTO3C transducer on a Crass Model 7 polygraph. The tissues were suspended in Ringer solution for one hour. They were then placed in calcium-zero Ringer solution for an additional hour. This was followed by the addition of norepinephrine (1 X 10^{-6}M) with and without hydralazine (1 X 10^{-5}M) for ten minutes. At the end of this time, $CaCl_2$ containing ^{45}Ca was added so that the final calcium concentration was 0.3 mM. The tissues were allowed to equilibrate for one hour, at which time they were removed from the water bath, blotted, weighed, ground in a tissue grinder, and an aliquot of the supernatant solution counted for radioactivity. Tissue calcium determinations were made by treating the tissues as above, without ^{45}Ca. They were then dissolved in nitric acid for twelve hours, diluted, and the tissue calcium determined by means of an atomic absorption spectrophotometer.

C. Results. In the presence of norepinephrine there was a significant 15% increase in the ^{45}Ca uptake. In the presence of hydralazine and norepinephrine there was a decrease in the ^{45}Ca uptake of 17%, as compared to controls with norepinephrine. There was approximately a 45% increase in the calcium content of aortas treated with norepinephrine (2.3 ± 0.17 meq/kg wet tissue in controls and 3.34 ± 0.31 meq/kg in norepinephrine-treated aortas). In the presence of hydralazine and norepinephrine there was a slight (not significant) increase in calcium content (2.50 ± 0.22 meq/kg). Hydralazine inhibits the tension induced by norepinephrine. This inhibition is dose determined. The greatest effect is seen after the initial contraction when there is marked relaxation in the presence of hydralazine. The inhibition by hydralazine can be completely reversed if LiCl is substituted for NaCl. Preliminary experiments indicate that the inhibition of ^{45}Ca uptake produced by hydralazine does not occur in the presence of LiCl.

Most recent work has been to study the effects of various
cationic changes and hydralazine on the outflow of calcium
from vascular smooth muscle. In these experiments the
isolated rabbit aortic strip is loaded with ^{45}Ca for varying
periods of time in the presence of norepinephrine (1 x 10^{-6} M).
The rate of calcium outflow is followed into a non-radioactive
solution containing various concentrations of calcium with
and without norepinephrine and hydralazine and in the presence
of various cationic changes. It has been possible to show
that in the presence of hydralazine there is a significant
15% increase in ^{45}Ca loss from the tissues which is accentuated
by the presence of LiCl substituted for NaCl. This effect
of hydralazine on calcium outflow is temperature sensitive
(inhibition by temperatures below 27°C). Further studies
are being performed to investigate this calcium effect in
more detail.

D. Discussion. We have interpreted the data to suggest that
    hydralazine has two effects on vascular smooth muscle.
    One effect is to decrease the change and increase the calcium
    permeability induced by norepinephrine. The second effect is
    that hydralazine appears to increase the calcium outflow from
    the vascular smooth muscle. This effect may be related to
    stimulation of an energy-dependent calcium-outward pump. Changes
    in hydrogen ion and potassium do not appear to affect this pump,
    but the substitution of sodium for lithium does appear to
    produce some inhibitory effect which suggests that the sodium
    ion and the calcium ion may compete for the outward transport
    system. We have not been able to determine whether this pump
    is coupled to an inward transport of cations. This data
    would support the concept that hydralazine may stimulate a
    calcium-outward pump and produce relaxation. Again, one might
    also speculate that since hydralazine is known to be a potent
    antihypertensive agent, it may be quite possible that certain
    diseased states associated with abnormal blood pressure, such
    as essential hypertension, may be related to abnormalities
    of relaxing systems rather than abnormalities of stimulating
    systems.

    For these reasons it is proposed to study patients and animals
    with abnormalities of blood pressure to determine (1) if there
    are circulating factors which affect relaxing systems and (2) if
    changes in relaxing systems play a role in the etiology or main-
    tainance of these abnormalities.

    These studies are to be carried out in isolated vascular smooth
    muscle strips and isolated relaxing systems from vascular smooth
    muscle.

Prior to investigation of the relaxing systems of vascular
smooth muscle, studies of the relaxing system of cardiac
muscle have and are being carried out. This is because (1)
the techniques to be used for vascular smooth muscle need to
be worked out first in systems where more tissue is available,
and yet offer similar problems in isolation and utilization,
(2) comparison of cardiac and vascular relaxing systems is
important, and (3) investigations of cardiac relaxing systems,
particularly the effects of electrical stimulation, are of
special interest.

II.   Isolated Cardiac Relaxing Systems

A.   Progress. We have continued to study the effects of electrical
stimulation on calcium outflow in cardiac sarcoplasmic reticulum
(relaxing factor).

B.   Methods. The sarcoplasmic reticulum is isolated and partially
purified by the use of a sucrose gradient ultracentrifugation.
These fragments are then incubated in an appropriate medium
containing ^{45}Ca in the presence or absence of specific pharma-
cologic agents. An aliquot of the medium is then placed on
a Millipore filter which has been adapted with platinum mesh
wire in order to study the effects of electrical stimulation.
Parameters have been defined as to the effect of voltage
intensity, stimulus duration and stimulus frequency on the
release of calcium by the fragments of the sarcoplasmic reticulum.

Recent evidence suggests that calcium is the link between
membrane depolarization and muscle contraction. It has been
proposed that during excitation calcium is released or made
available, possibly from sites in the sarcoplasmic reticulum,
thus increasing the intracellular calcium concentration in
the vicinity of the myofibrils, resulting in a contraction.
Following contraction, calcium is rebound and relaxation takes
place. It is hoped that studies of this nature will afford
further insight into the factors affecting calcium uptake and
release as well as the subcellular mechanisms of action of
various pharmacologic agents.

Calcium outflow studies are done with grana filled with ^{45}Ca layered
on a Millipore filter with platinum mesh electrodes above and
below (Figure 1). The basic non-radioactive outflow solution
consists of a buffer, 100 mM KCl, and 1×10^{-4} EGTA. This solution
is modified for different experiments. Outflow solution is
added, and, at varying intervals of time, the amount of ^{45}Ca
appearing in this solution is counted. It has been found that

electrical stimulation increases calcium outflow. This effect
depends upon voltage, stimulus duration, and frequency.
Lanthanum at low concentrations (1.8×10^{-5} M, 4×10^{-4} M) decreases
calcium outflow from sarcoplasmic reticulum during the non-
stimulated state. At low concentrations it has little effect
on the calcium released during electrical stimulation, but can
inhibit calcium release caused by electrical stimulation at
higher concentrations (1.8×10^{-3} M). These effects can be
seen in Figures 2 and 3. Figure 2 is a plot of the percent of
^{45}Ca retained in the grana with respect to time, with and without
stimulation and lanthanum. Note that electrical stimulation
decreases the amount of calcium retained in the sarcoplasmic
reticulum. Lanthanum at a concentration of 1.8×10^{-4} M
significantly increases the amount of ^{45}Ca retained in the
sarcoplasmic reticulum. Electrical stimulation in the presence
of lanthanum at this concentration does cause a decrease in
the calcium remaining in the sarcoplasmic reticulum. It has
been found that either using low concentrations of lanthanum
or pre-treating the sarcoplasmic reticulum with high concen-
trations of lanthanum for a short period of time will decrease
the resting calcium permeability and allow one to stimulate
electrically for long periods of time. This can be seen in
Figure 3. In this experiment the grana, while on the filter,
are treated with lanthanum 1.8×10^{-3} M for 8 minutes.
Electrical stimulation is applied at 8 minutes, 18 minutes, and
28 minutes. Note that there is a significant increase in
calcium released; the percent released returns toward baseline
levels after each stimulation and the maximum percentage
released decreased with multiple stimulations, as one would
expect. Another interesting aspect of the effects of electrical
stimulation can be seen in this figure, and that is that when
the electrical stimulation is turned off (after 2 minutes of
stimulation) there is a decay in the effect of electrical
stimulation on calcium outflow which has a half-life of approx-
imately 2.2 minutes. This is interesting in light of the fact
that the time constant for an increase or decrease in the
inotropic state of a resting cardiac muscle is long (half life
of about 100 seconds) (Circulation Research 24:409-445, 1969).

Another study is being performed to determine the effects of
changes in frequency in the range which produces increased con-
tractile tension in cardiac muscle and its effect on calcium
release. Experiments indicate that there is a significant
increase in calcium release from cardiac grana when the stimulus
is changed from 0.5 cps to 1.0 cps and to 2.0 cps (Figure 4).
In this figure we have plotted the percent calcium released with
respect to time and the effects of changing the stimulus frequency

from 0.5 to 2.0 cps. Note that there is an increase in the
release of calcium from approximately 14% to 20% when the
frequency is changed from 0.5 cps to 1.0 cps. There is a
further rise to 24.5% when the frequency is changed to 2.0 cps.
This data suggests that the frequency potentiation seen in a
variety of muscle, including rabbit heart muscle, from 0.5 cps
to 2.0 cps may be related to an increase in calcium release
from sarcoplasmic reticulum. In addition, it is well known that
pentobarbital can inhibit the frequency potentiation in cardiac
muscle. Figures 5 and 6 show the effects of pentobarbital on
electrical stimulation. In Figure 5 we have plotted percentage
of calcium released with respect to time. Note that during the
first stimulation at 8 minutes there is approximately a 19.5%
^{45}Ca release. The grana are then treated with pentobarbital
at a concentration of 4 mM. A second stimulation in the presence
of pentobarbital only releases 11.5%. Following this, the
pentobarbital is washed out and a third stimulation is done at
20 minutes. Note that there is approximately an 18% release
during that interval. Thus, it can be seen that pentobarbital
inhibits the release of calcium during electrical stimulation,
and this effect is reversible. In Figure 6 the grana have been
exposed to 4 mM pentobarbital, stimulated for a period of 15
seconds during the fourth 2-minute interval, and the pentobarbital
is then washed out. The grana are then stimulated on the seventh
2-minute interval and the pentobarbital is added again and the
grana are stimulated for a third time on the tenth 2-minute
interval. Note that there is an inhibitory effect on ^{45}Ca
release in the presence of pentobarbital. When the pentobarbital
is removed, the release of calcium is markedly increased. The
effects of pentobarbital are dose dependent and can be seen in
concentrations of approximately 0.1 to 4 mM. These studies
suggest that the effects of pentobarbital on the frequency
potentiation in cardiac muscle are related to an inhibition of
electrical release of calcium from sarcoplasmic reticulum or
other sites in the cardiac cell. Further studies investigating
the effects of cardiac glycosides, catecholamines, and carbachol
in these parameters are being carried out.

Another study is being conducted to determine the effects of
paired stimuli on calcium release from sarcoplasmic reticulum.
In these studies the stimulus frequency is maintained at 0.5
or 1.0 cps. If a paired stimulus is placed 200 msec following
initial stimulation there is an increase in calcium outflow
beyond that which is seen at a stimulus frequency of 1.0 cps
or 2.0 cps (Figure 4). In this figure it can be seen that, at
a stimulus frequency of 1.0 cps, approximately 20% of the total
^{45}Ca is released. When the stimulus frequency is raised to
2.0 cps there is an increase to 24.5%. However, if during this
interval the same number of beats occur but are paired so that

the second beat occurs 300 msec after the initial stimulation, there is a further increase in calcium outflow up to approximately 28%. It should be pointed out that in these experiments the grana are only stimulated for 15 seconds during the 2-minute interval. Further studies are being carried out in which the grana are stimulated for longer intervals in an attempt to potentiate these effects. The studies suggest that the potentiation of tension seen in cardiac muscle with paired stimuli could also be explained on the basis of an additional release of calcium from the sarcoplasmic reticulum or other membranous sites. Further studies are being conducted on the effects of pentobarbital and carbachol on paired stimuli and the effect on calcium release from sarcoplasmic reticulum.

In addition to more complete studies of calcium release during electrical stimulation from sarcoplasmic reticulum, we are also in the process of investigating calcium uptake and the effects of electrical stimulation. In these experiments grana are placed on Millipore filters and the calcium uptake, both active (in the presence of oxalate) or non-active binding will be examined with the grana on the filter, with and without electrical stimulation. These studies are being done because it may be possible that a variety of cardiotonic agents may affect calcium release indirectly by changing calcium uptake, either active or non-active during electrical stimulation. To indicate that these experiments are feasible and that the active calcium transport system is not disturbed by electrical stimulation, we have included Figure 7. In these experiments the grana are placed on Millipore filters and stimulated for a period of 15 seconds. In the controls no stimulation was given. Following this ATP and Mg (5 mM) potassium oxalate, 0.1 mM $CaCl_2$ and Ca were added and the uptake was started. At various intervals of time the reaction was stopped by suction, and an aliquot of the filtrate was counted, as well as the filter. It can be seen that there is an increasing uptake of calcium by the grana over a 30 minute period and that prior electrical stimulation has no effect on this parameter.

It is felt that with the techniques that have been developed to study electrical stimulation of sarcoplasmic reticulum will be extremely valuable in elucidating the mechanism of action of a variety of drugs on cardiac muscle. With these techniques we also feel that it will be equally important in investigating relaxing systems in vascular smooth muscle. I believe this simple technique will result in new and exciting discovery of drug action.
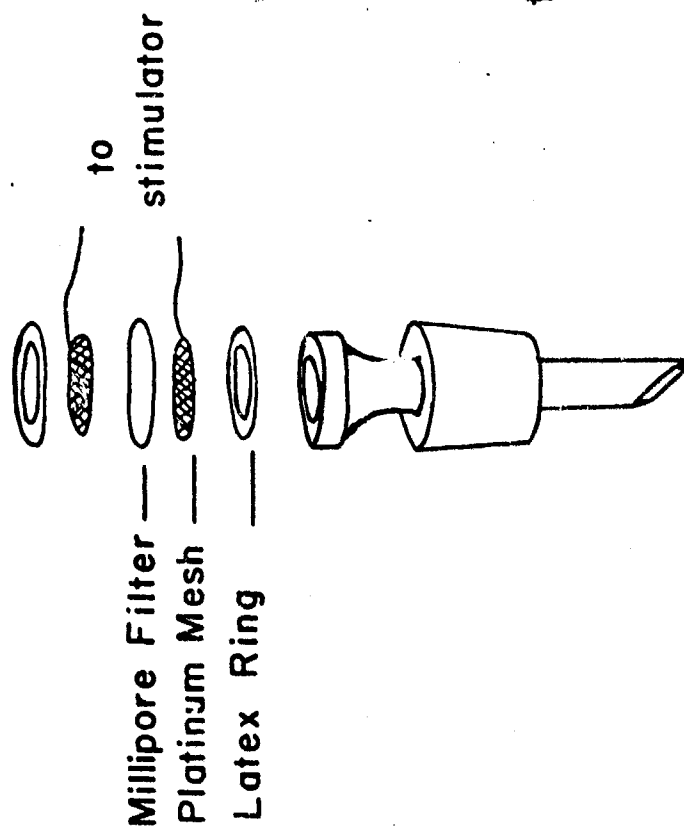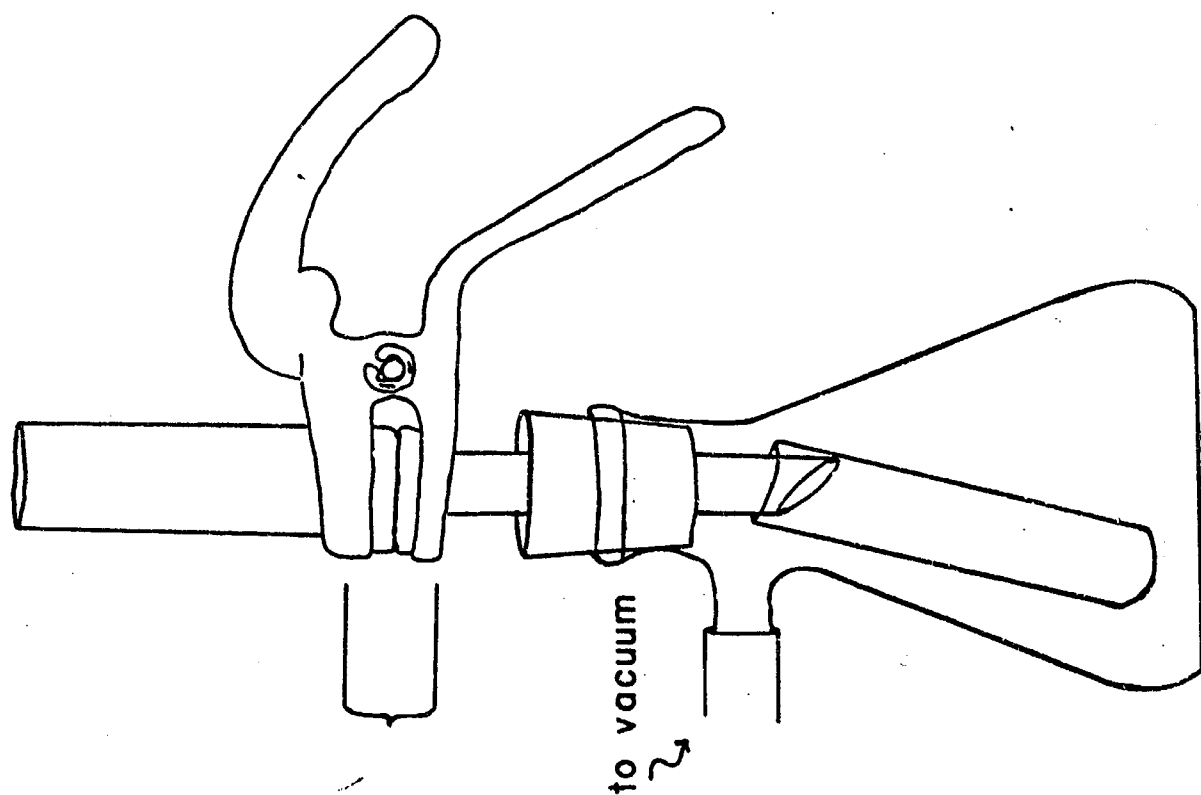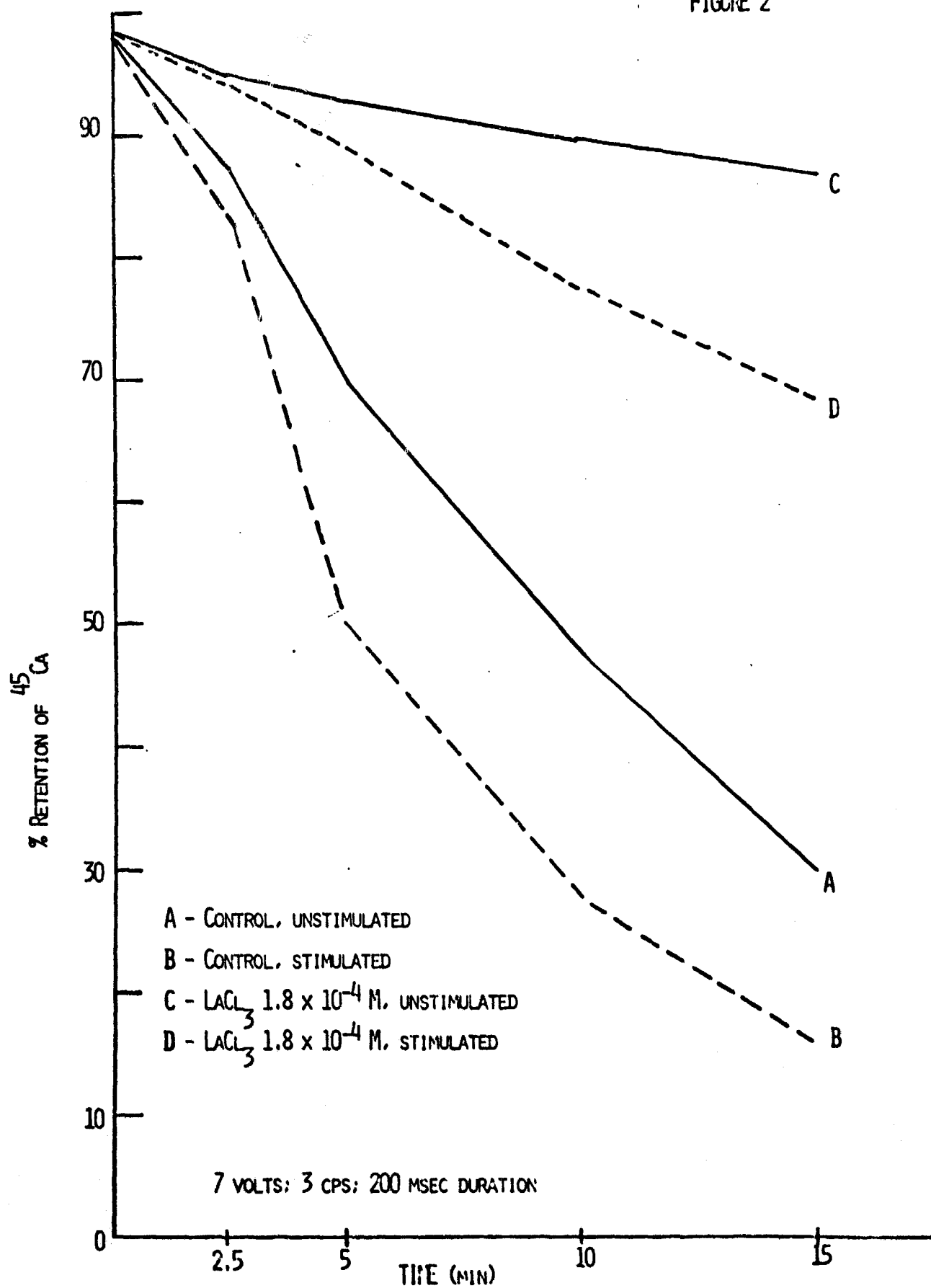
FIGURE 1

to stimulator

to vacuum

Millipore Filter
Platinum Mesh
Latex Ring

FIGURE 2

A – Control, unstimulated
B – Control, stimulated
C – LaCL$_3$ 1.8 x 10^{-4} M, unstimulated
D – LaCL$_3$ 1.8 x 10^{-4} M, stimulated

7 volts; 3 cps; 200 msec duration

FIGURE 3

4.25 VOLTS
1 CPS
300 MSEC DURATION
LaCl$_3$ 1.8×10^{-3} M

FIGURE 4

- X—X CONTROL
- •—• 0.5 CPS
- △—△ 1.0 CPS
- ■—■ 2.0 CPS
- O—O 1.0 CPS WITH PAIRED STIMULUS, 300 MSEC DELAY

8 VOLTS
100 MSEC DURATION

% OF TOTAL RELEASE OF ^{45}CA

STIM

TIME (MIN)

FIGURE 5

.8 VOLTS
1 CPS
100 MSEC
   DURATION

FIGURE 6

Legend:
- ●———● UNSTIMULATED
- ——— STIMULATED
- 8 VOLTS
- 1 CPS
- 100 MSEC DURATION

Y-axis: % OF TOTAL RELEASE OF ^{45}CA

X-axis: TIME (MIN)

PENTOBARBITAL   PENTOBARBITAL

STIM   STIM   STIM
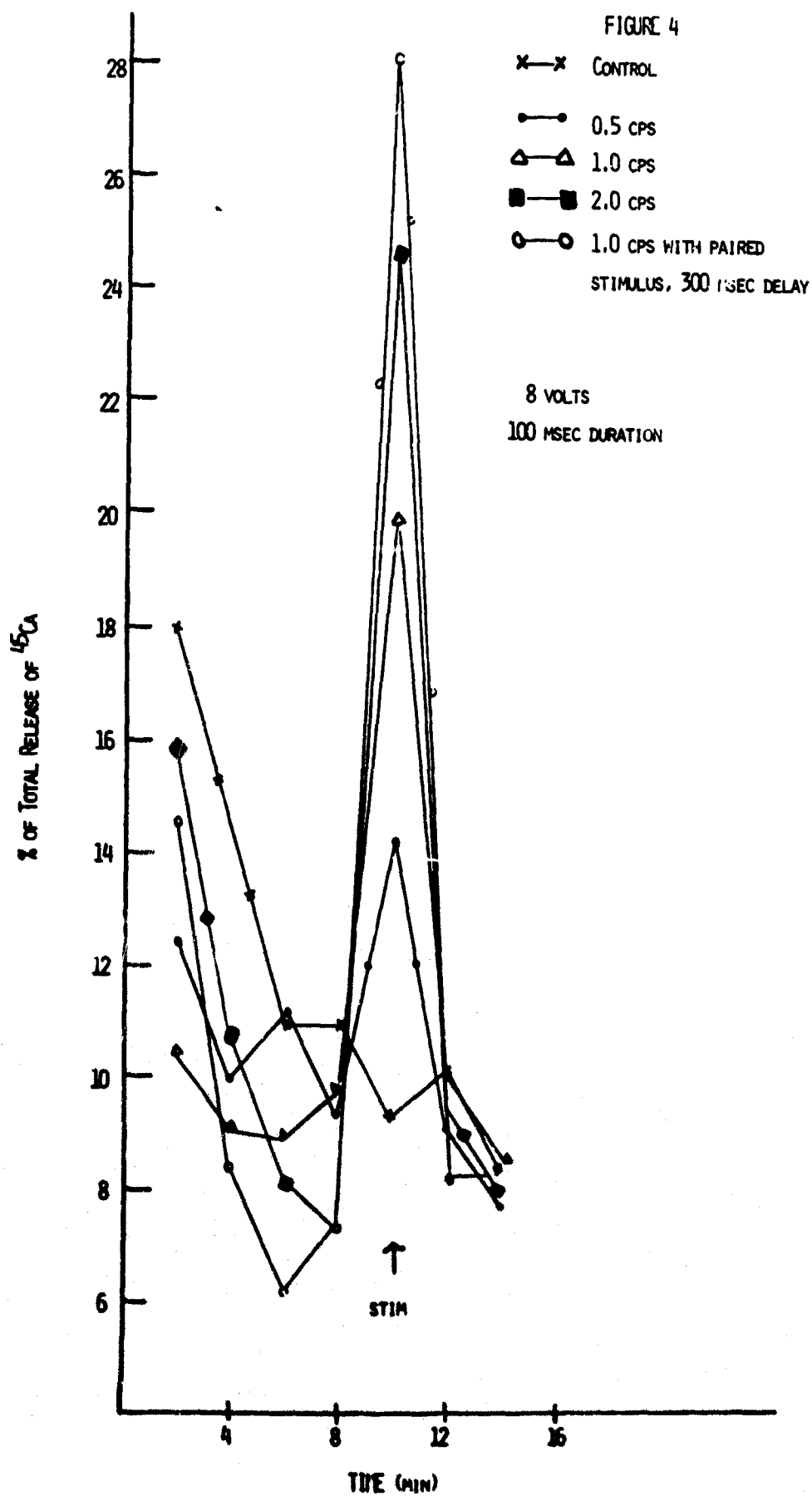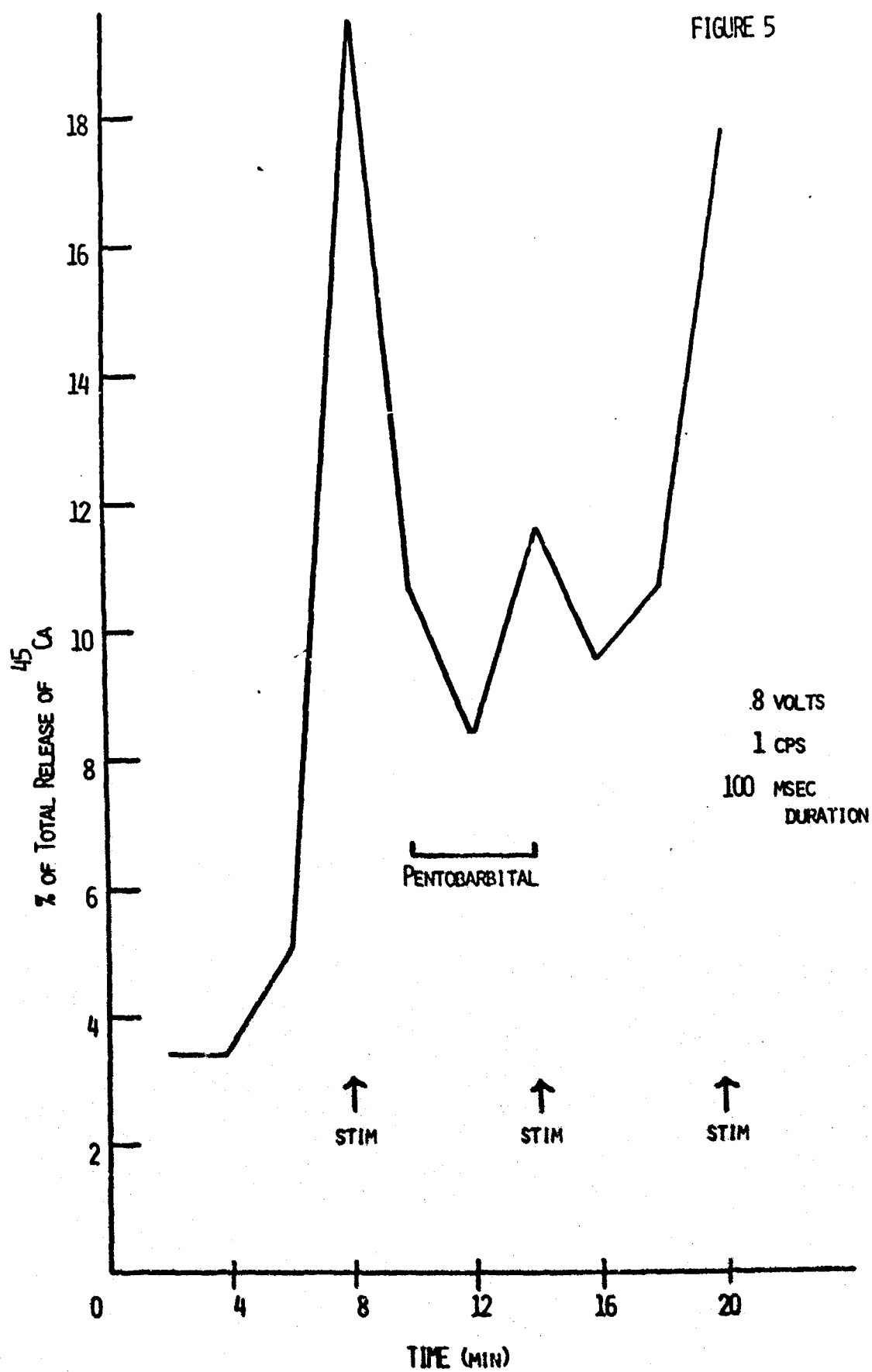
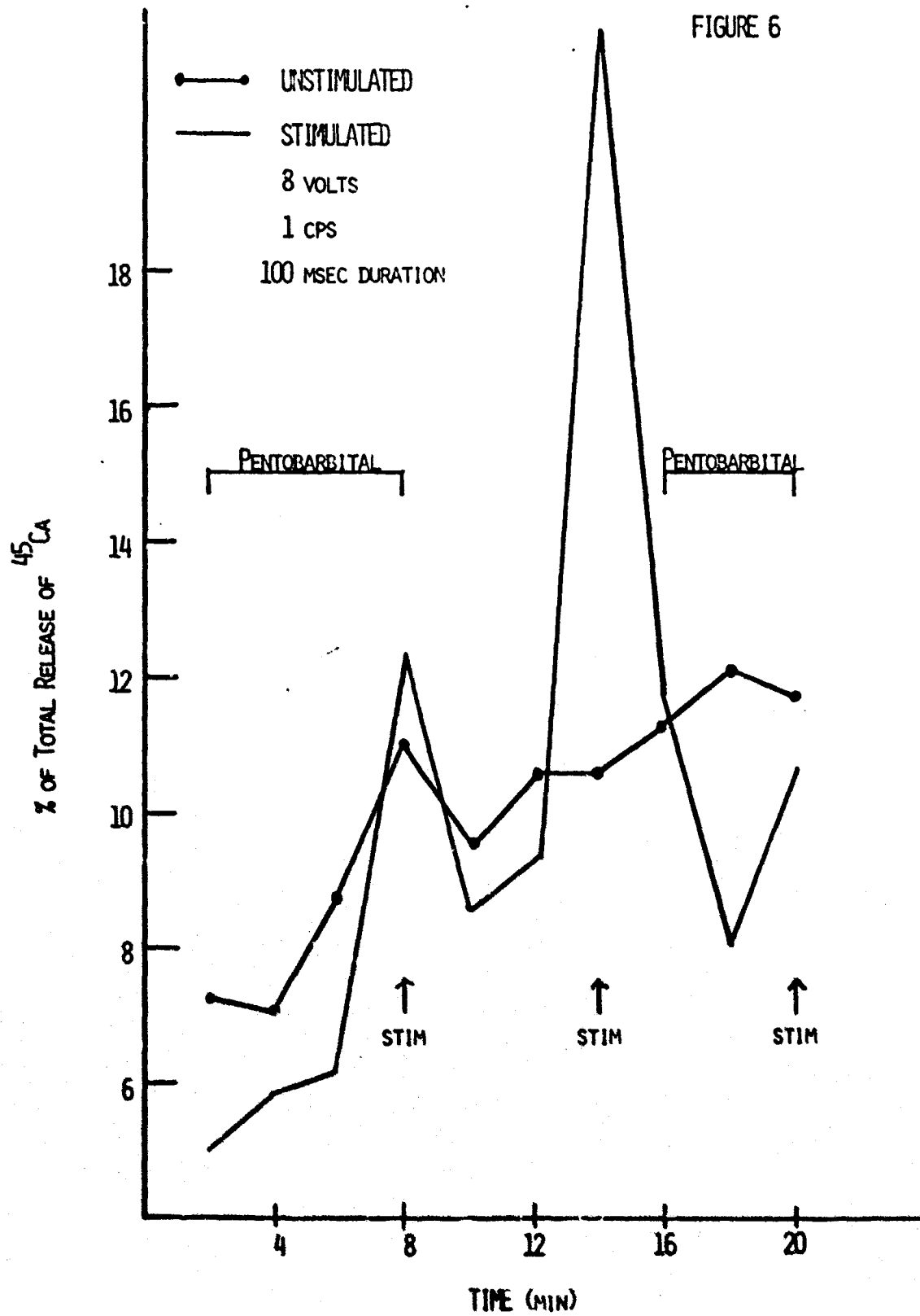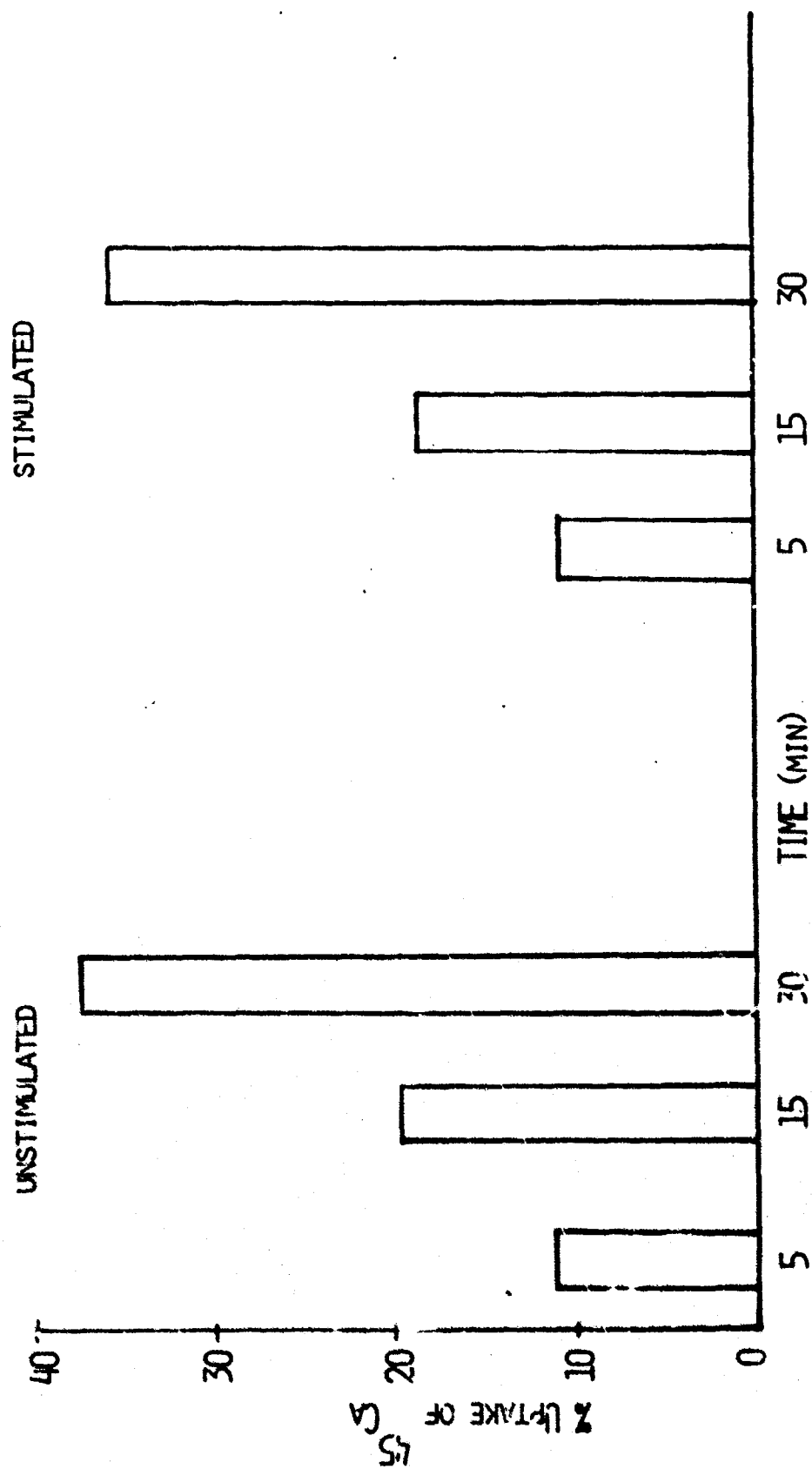FIGURE 7

I.   The cardiac effects of curare (See reprint of publication of this
     work which is attached). We completed this work during this period.

II. The acute diuretic response of guanethidine and reserpine. This
     work was completed and has been accepted for publication by
     Archiv. Int. Pharmacodyn. (See attached publication).

III. Responses of isolated rabbit atria to calcium. The initial phase
     of this work has been completed (See manuscript attached). In this
     study we were able to show that rabbit atria were more sensitive
     to calcium 4 hours but not 24 hours after 3 mg/kg reserpine.
     Changes in heart function which occurred were: (a) a decrease in
     the threshold response to calcium, (b) an increase in the rate of
     change of tension development, (c) a greater incidence of calcium-
     induced arrhythmias, and (d) a decrease in the rate of decline in
     tension after removal of calcium from the medium. We found no
     change in heart rate occurring during these experiments. Also,
     propranolol, a beta adrenergic blocking agent failed to effect the
     results. We have concluded from this that reserpine probably increases
     membrane permeability to calcium, and that it may also alter the
     intracellular calcium (relocate or change its binding characteristics).

         This work is still in progress. The phase current is to study
     any differences in our findings that occur in the electrically driven
     atria and the spontaneously beating preparation. At this point we
     have found none.

IV. The effect of change in environmental (perfusate) temperature on
     vascular tone. This study has been completed (See attached abstract

and manuscript) and led to the Doctoral Dissertation of James C.

Murphy (Title: SUPERSENSITIVITY IN VASCULAR SMOOTH MUSCLE INITIATED

BY COLD).

We found that blood vessels (dog femoral and rabbit aortas) were

supersensitive to catecholamines after exposure to cold. A study of

electrolyte shifts during exposure led to the conclusion that some

change in calcium of the tissue was the underlying cause.

V. A study of sodium pump reversal. This study is far from complete

and the data have only led to confusion in interpretation so far.

We have seen the sodium pump reversal when vascular tissue is incu-

bated in cold (4°C) or room temperature or at 37°C. This is charac-

terized by a doubling of the sodium content of the tissue, a drastic

reduction in potassium (at times to levels too low to detect).

This process can be accelerated by a low calcium environment, and

can be partially blocked by ouabain or anoxia. Drugs which cause

contraction (norepinephrine, vasopressin, angiotension) have no

effect. The study will continue.

The Effects of d-Tubocurarine and Its Commercial Vehicles on Cardiac Function

Oliver Carrier, Jr., Ph.D.,* and James C. Murphy, M.S.†

d-Tubocurarine has been reported to have a depressant effect on cardiac tissues, an effect reversed by high calcium concentrations. Results of this study showed that the depressant effect (20 to 50 per cent of control tension) could be accounted for by the benzyl alcohol or 4-chloro-3-methyl cresol used as preservatives in commercial preparations of d-tubocurarine. The effects of these compounds were reversed by increasing the calcium chloride content of the Ringer's solution 25 to 100 per cent (3.0 to 4.8 mM). The findings of previous workers may have reflected the effects of these two substances rather than a depressant effect of the d-tubocurarine. (Key words: d-Tubocurarine; Cardiac function.)

IT HAS BEEN REPORTED that d-tubocurarine has a cardiac depressant effect [1,3] which can be reversed by high calcium concentrations.[4] Some investigators believe this depressant effect is the result of histamine release by d-tubocurarine.[1,2] Others claim a direct cardiac action for the drug.[5,6] In our studies we have been interested in involvement of calcium ion in vascular and cardiac muscle function. We have used reserpine to modify vascular calcium content and, we believe, availability to the contractile apparatus.[6,7] We thus became interested in the possibility of a curare–reserpine–calcium interaction at various muscle sites. In one study [8] we found that reserpine decreased the apparent potency of d-tubocurarine at the skeletal neuromuscular junction. We, therefore, decided to study this relationship in the heart. However, in the initial phase of the study we were unable to demonstrate any cardiac depressant activity with d-tubocurarine, nor any calcium–curare relationship in the isolated rabbit atrial preparation. Further work to resolve this difference between our results and those of the previous workers was needed. The results of this study are the subject of the present report. The cardiac depressant effect which had been attributed to d-tubocurarine resulted from the preservatives used in the injection preparations of the drug, and d-tubocurarine, *per se*, had none.

Methods

EXPERIMENTAL PROCEDURES

Albino New Zealand rabbits weighing approximately 1 kg were used in the study. Each animal was sacrificed by a sharp blow to the head, and the heart was excised immediately. The hearts were placed in oxygenated Ringer's solution and the atria removed. Atria were suspended in organ baths (50 ml) in Ringer's solution (composition: NaCl, 154 mM; KCl, 5.4 mM; $CaCl_2$, 2.4 mM; NaHCO$_3$, 6 mM; dextrose 11 mM; distilled water to 1 liter). Tension measurements were made with "E and M" myographs and recording equipment (Physiograph) after adjusting the diastolic tension of the atria to 1 gram. Three preparations were used in these studies: the spontaneously-beating right atrium, spontaneously-beating left and right atria, and the electrically-driven left atrium. Left atria were stimulated at a frequency of 1/sec with supramaximal square-wave stimulation 3 msec in duration. Recording of contractile amplitude and rate was begun immediately after the atria were mounted. A 30-minute equilibration period preceded the final adjustment to 1-gram tension, and a second 30-minute equilibration period followed prior to the addition of drugs or calcium. After the equilibration period, d-

tubocurarine was added to the bath in the concentrations indicated (see "Results"). After sufficient time had elapse for any changes due to the d tubocurarine to occur, calcium chloride was added. Changes in contractile amplitude and rate were used to assess the effects of the drugs. One series of rabbits was pretreated 24 hours before the experiment with 3.5 mg/kg reserpine. All solutions of d-tubocurarine were tested for paralytic activity in unanesthetized rabbits before use in the *in vitro* experiments.

Drugs

The commercial drugs used were the injectable preparations of d-tubocurarine: Abbott's *tubocurarine chloride*, containing 3 mg d-tubocurarine chloride, 1 mg sodium metabisulfite,

9 mg benzyl alcohol and 1 ml of water made isotonic with NaCl; Burroughs Wellcome's *Tubarine*, containing 3 mg d-tubocurarine chloride, 1 mg p-chloro-m-cresol (4-chloro-3-methyl-phenol) and 1 mg potassium metabisulfite in 1 ml of water made isotonic with NaCl; Squibb's *tubocurarine chloride*, containing 3 mg d-tubocurarine chloride, 9 mg benzyl alcohol and 1 mg sodium bisulfite in 1 ml of water made isotonic with NaCl. Also used were the pure crystalline d-tubocurarine chlorides obtained from Abbott, Burroughs Wellcome, Squibb, and Nutritional Biochemicals. Other agents used were reagent-grade benzyl alcohol and 4-chloro-3-methyl-phenol. Drug vehicles used were: Abbott 3386, containing 9 mg benzyl alcohol, 1 mg sodium metabisulfite and 4.6 mg NaCl in 1 ml of water; Ab-
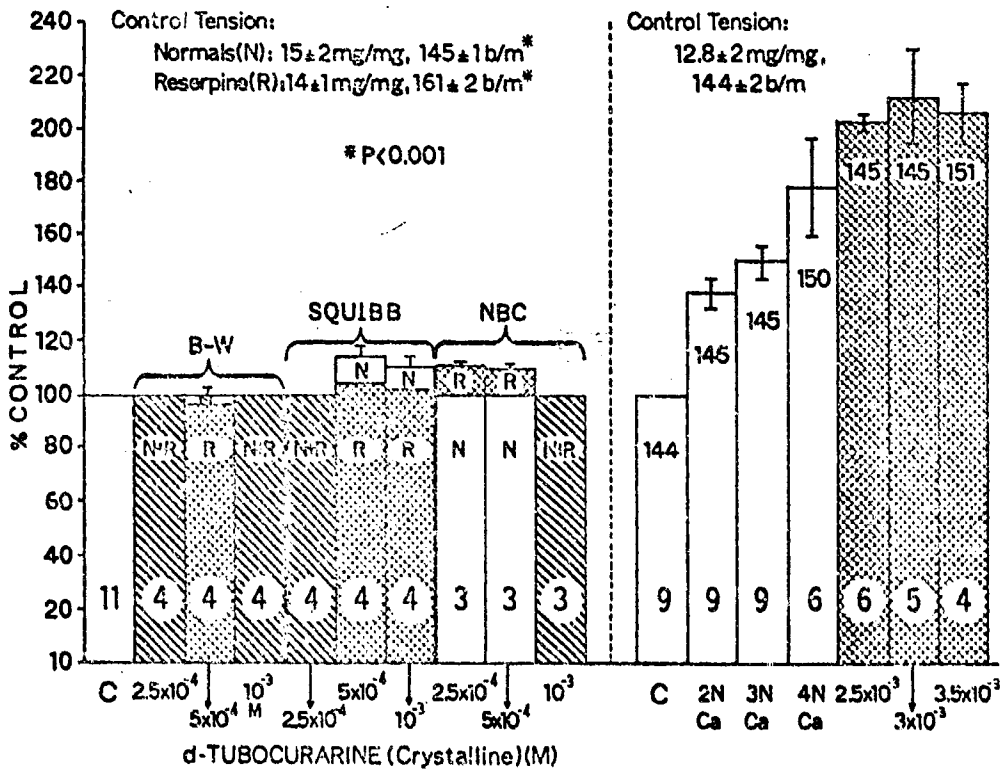
Fig. 1. Effects of d-tubocurarine on contractile tension of unstimulated rabbit left and right atria. Left side of figure: C, control; N + R, normal and reserpine-treated atria superimposed; R, reserpine-treated atria; N, normal atria; B-W, Burroughs Wellcome; NBC, Nutritional Biochemicals. Ordinate, contractile tension as per cent of control; abcissa, molar concentration of d-tubocurarine. Right side of figure: Shaded bar, control; clear bars after addition of calcium; cross-hatched bar, after both high calcium concentration (4 N) and d-tubocurarine. Ordinate, contractile tension as per cent of control; abcissa, concentration of calcium added as multiples of normal (N = 2.4 mM), a molar concentration of d-tubocurarine. Numbers in lower parts of bars represent the numbers of atria tested, those in upper parts (right side) represent heart rates.
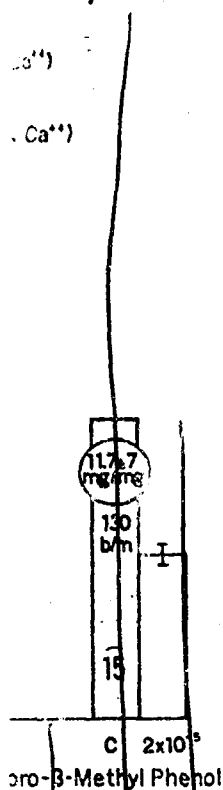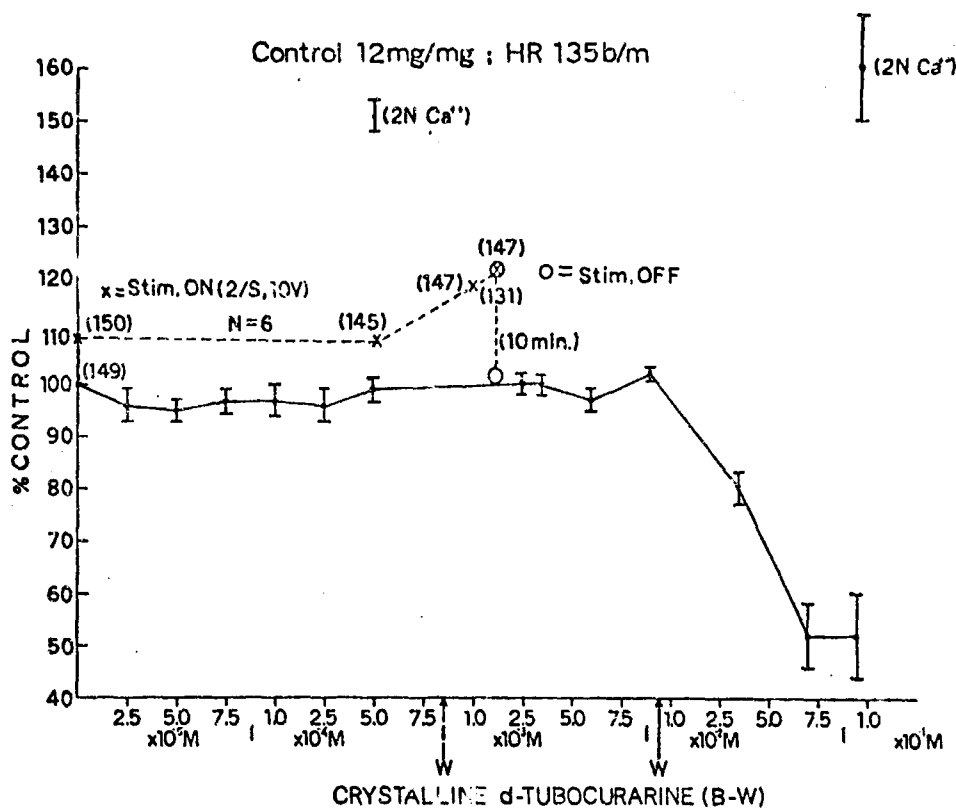
FIG. 2. Effects of d-tubocurarine on the stimulated and unstimulated rabbit left and right atrial preparation. Solid line, unstimulated; dashed line, stimulated. Ordinate, contractile tension as per cent of control; abcissa, molar concentration of d-tubocurarine (Burroughs Wellcome). The result of calcium addition is shown by two points off the curve. The preparation was washed at W. Vertical bars, standard errors of the means. Six atrial preparations were tested.

bott 3S41, containing 1 mg sodium metabisulfite and 6.8 mg NaCl in 1 ml of water; Burroughs Wellcome placebo (injection), containing 1 mg potassium metabisulfite and 1 mg methylparaben (P-hydroxybenzoic acid methylester) per ml of water made isotonic with NaCl. All drugs and calcium chloride were made up in concentrated stock solution for use, or taken directly from original vials. Delivery of drugs or calcium to the organ bath was in as small a volume as possible, depending upon the maximum concentration of the drug that could be put in solution. In most instances the volume did not exceed 0.1 ml. Periods of 15 to 30 minutes were allowed between drug or calcium additions to the organ baths. All baths were oxygenated with a mixture of 95 per cent O_2 and 5 p. Temperature was maintained at 37 ± , C,

and pH was maintained at 7.4 ± 0.5. At times, small quantities of HCl or NaOH had to be added to the baths to adjust pH.

Results

EFFECTS OF CRYSTALLINE d-TUBOCURARINE ON ATRIA FROM NORMAL AND RESERPINE-TREATED RABBITS

The results obtained when isolated, unstimulated atria from control and reserpine-treated (3.5 mg/kg 24 hours prior to the experiment) rabbits were subjected to crystalline d-tubocurarine from 2.5×10^{-4} M to 10^{-3} M are depicted by the bar graph on the left in figure 1. There were no significant changes in contractile tension in either control or reserpine-treated atria when d-tubocurarine was added. When the calcium content of the Ringer's solution was raised to four times normal (9.6 mM
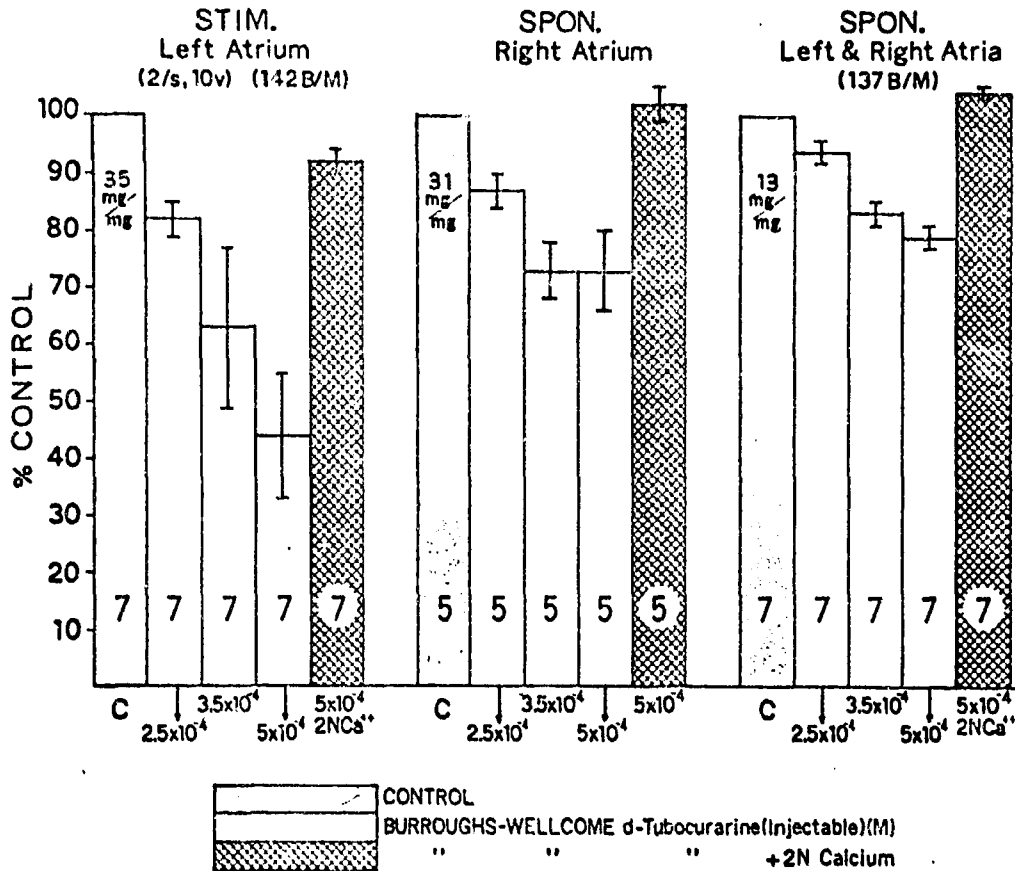
Fig. 3.   Depressant effect of injectable d-tubocurarine preparations and reversal by the addition of calcium.   Control tension is given in the first bar for each preparation.   Heart rates are shown at the top.   The rate of the spontaneous right atrium was not significantly different from that of spontaneous left–right atria.   Ordinate, contractile tension as per cent of control; abcissa, molar concentration of d-tubocurarine.   Vertical bars, standard errors of the means.

final concentration) there was a significant increase in contractile tension of normal atria. d-Tubocurarine $(2.5-3.5 \times 10^{-3}$ M) had no significant effect on the tension of these atria in the presence of high calcium concentrations (bar graph on the right in figure 1).   In figure 2 we show the results of an experiment to test the effect of stimulation on the left and right atrial preparation treated with d-tubocurarine $(2.5 \times 10^{-5}-10^{-1}$ M).   (Previous investigators reporting curare depression used the stimulated left atrial preparation.[4])   Stimulation at 2/sec (10 v) increased contractile tension about 10 per cent; however, d-tubocuraine had no depressant effect on either preparation up to 10^{-3} M.   From 10^{-3} M d-tubocuraine to 10^{-1} M, a progressive decline in tension was observed.   The addition of two

times normal calcium (final concentration three times normal, 7.2 mM, $CaCl_2$) increased the tensions of both stimulated and unstimulated atrial preparations (41 and 52 per cent, respectively) in the presence of 5×10^{-3} M tubocurarine.   The addition of 2 N calcium in the presence of 10^{-1} M d-tubocurarine increased atrial tension from 52 per cent to 160 per cent of control.

EFFECTS OF INJECTABLE d-TUBOCURARINE ON THE CONTRACTILE TENSION OF RABBIT ATRIA

A depressant effect of Burroughs Wellcome injectable d-tubocurarine on contractile tension was found in the stimulated left atrial, spontaneous right atrial, and spontaneous left and right atrial preparations (fig. 3).   There

was a direct relationship between the depression observed and the concentration of drug used for each preparation. The stimulated left atrial preparation was depressed far more than either of the spontaneous preparations. In the latter there was no further significant depression at concentration of d-tubocurarine above 3.5×10^{-4} M. In each case when the calcium content of Ringer's solution was increased by twice the normal concentration (final concentration three times normal, 7.2 mM CaCl$_2$) the depression was completely reversed. In figure 4 the depressant effect of Abbott's injectable d-tubocurarine on contractile tension is shown. These results are similar to those obtained with the Burroughs Wellcome product. Similar experiments with Squibb's injectable d-tubocurarine produced similar results. In figure 4 results obtained with the two vehicles for d-tubocurarine, Abbott 3386 and 3841, are illustrated. Vehicle 3386 had a depressant effect similar to that of the injectable tubocurarine, while 3841 did not. The depression resulting from 3386 was reversed by calcium. The depression by 3386 obtained upon the addition of 1.0 ml to the bath was not significantly different from that obtained with 8×10^{-5} M d-tubocurarine (final bath concentration upon the addition of 1 ml of the injectable drug to the bath). The vehicle used for Abbott's injectable tubocurarine chloride in these experiments was identical to 3386.

EFFECTS OF BENZYL ALCOHOL AND 4-CHLORO-3-METHYL PHENOL ON ATRIAL TENSION

The depressant effects of Squibb's and Abbott's d-tubocurarine on atrial tension resulted from the benzyl alcohol contained in their vehicles (fig. 5). Benzyl alcohol had the depressant effect previously accredited to the d-tubo-
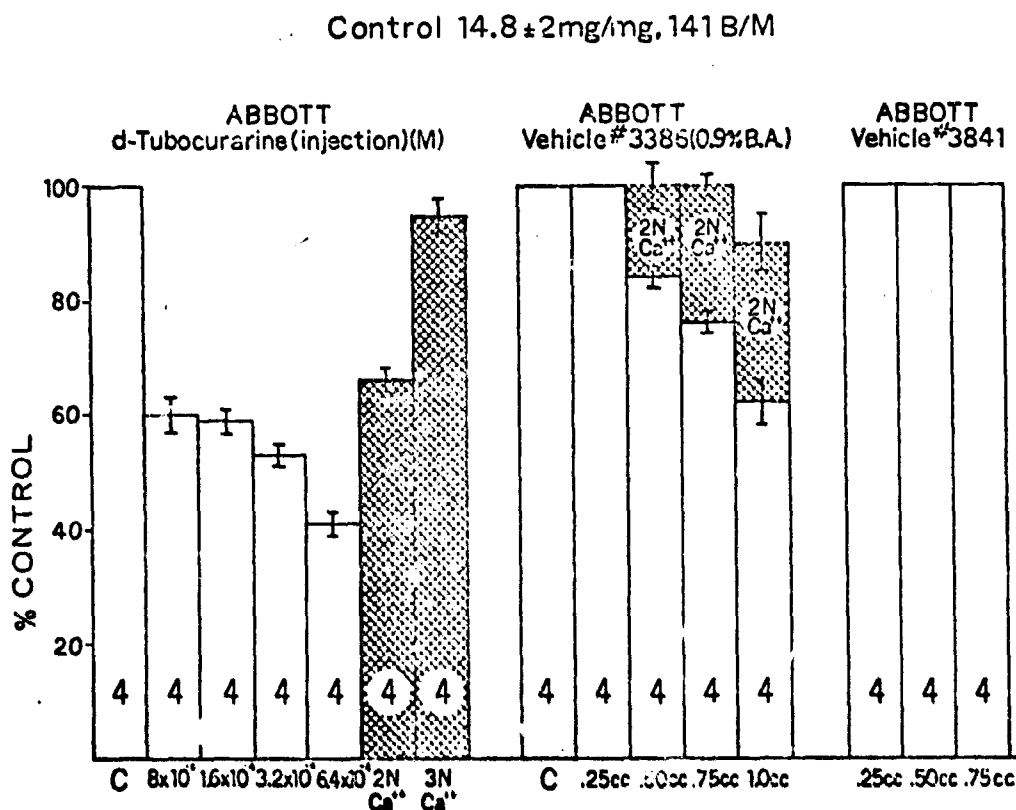
FIG. 4. Depressant effects of Abbott's injectable d-tubocurarine and vehicles 3386 and 3841 and calcium reversal. Control tension and rate at top of figure. Ordinate, contractile tension as per cent of control; abcissa, molar concentration of d-tubocurarine, calcium concentration as multiple of normal (N = 2.4 mM), and volume of vehicle delivered. Numbers at lower parts of bars, numbers of atria tested; verticle bars, standard errors of the means.
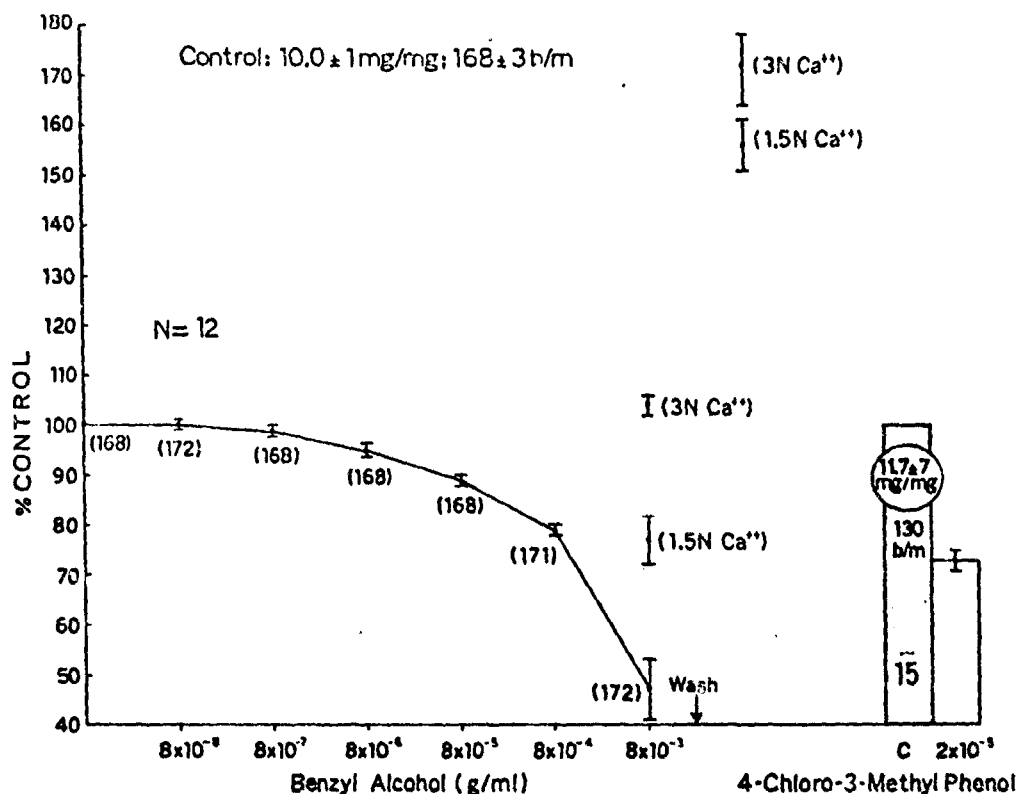
FIG. 5. Depressant effects of benzyl alcohol and 4-chloro-3-methyl phenol on the unstimulated left and right rabbit atrial preparation. Reversal by the addition of calcium is shown by isolated points in the figure both before and after washing (at "wash" arrow). Control rate and tension for atria tested with benzyl alcohol are given at the top, for those tested with the phenol, rate and tension are shown in control bar. Numbers in parentheses under the curve indicate heart rate. N, number of atria. Number in lower part of shaded bar, number of atria. Ordinate, contractile tension as per cent of control; abcissa, concentrations of benzyl alcohol and phenol in g/ml.

curarine. The active preservative in the Burroughs Wellcome product is 4-chloro-3-methyl phenol. When tested in 15 preparations, it reduced the tension of the spontaneous right and left atrial preparation 30 ± 5 per cent at 2 × 10^{-5} g/ml. The addition of calcium to as much as two times normal reversed both the benzyl alcohol and the phenol depressions.

EFFECTS OF ADDITION OF BENZYL ALCOHOL TO ATRIAL PREPARATIONS AFTER ADDITION OF CALCIUM

Experiments were done to determine the effects of pretreatment of the isolated atria with high calcium concentrations prior to the addition of benzyl alcohol, and benzyl alcohol plus d-tubocurarine. Results of these experiments are presented in table 1. At 1.25 nor-

mal calcium (final concentration), 10^{-7} to 10^{-6} g/ml benzyl alcohol reversed slightly the positive inotropic effect of the calcium. At 1.5 and 2.0 normal calcium, benzyl alcohol did not overcome the calcium response. At 2.5 normal calcium, the addition of the alcohol resulted in arrhythmias in all instances, so that the experiments were terminated. Addition of 1 mg of d-tubocurarine with each mg of benzyl alcohol did not affect these results. In these experiments the increase in calcium caused an increase in heart rate as well as an inotropic response. At 1.25 normal calcium, 10^{-5} g/ml benzyl alcohol brought the rate back to control from an increase of 25 per cent above control. At 1.5 normal calcium, benzyl alcohol only decreased the rate toward normal, about 10 per cent. At higher calcium concen-

TABLE 1. Effects of Benzyl Alcohol on Atrial Preparations after the Addition of
Calcium (Control Tension 16 ± 1 mg/mg; Control Rate 137 ± 2 beats/min)

Number of Atria Tested	Calcium Concentration Times Normal (2.1 mM)	Change after Calcium		Change after Calcium + Benzyl Alcohol*	
		Tension (Per Cent)	Rate (Per Cent)	Tension (Per Cent)	Rate (Per Cent)
7	1.25	+45 ± 1	+25	−55 ± 1†	−25†
4	1.50	+85 ± 3	+25	−50 ± 10	−10
4	2.0	+120 ± 5	+26	−30 ± 8	0
4	2.50	+240 ± 6	+22	Arrhythmias	0

* Atria were subjected in each instance to 10⁻⁷ to 10⁻⁴ g/ml benzyl alcohol, with the same result obtained at all concentrations. This amount of benzyl alcohol is equivalent to 1.1×10^{-3} to 1.1×10^{-6} M d-tubocurarine. Results obtained with benzyl alcohol plus crystalline d-tubocurarine (1 mg for each mg benzyl alcohol) after calcium were not significantly different from those obtained without the d-tubocurarine.

† Percent reduction in tension and rate from increased level caused by calcium addition, i.e., benzyl alcohol reduced the rate to control level when added after 1.25 N calcium chloride.

trations benzyl alcohol had no effect. The decrease in rate observed after the addition of the benzyl alcohol to the high-calcium-concentration solution could be restored to the initial high level (25 per cent above control) by the further addition of 0.5 normal calcium (1.2 mM).

EFFECTS OF BURROUGHS WELLCOME VEHICLE CONTAINING NEITHER BENZYL ALCOHOL NOR 4-CHLORO-3-METHYL PHENOL.

In 20 experiments with the spontaneous left and right atrial preparation this vehicle had no effect on either atrial rate or tension in volumes equivalent to the volumes of the Burroughs Wellcome injectable d-tubocurarine, which contained 10⁹ to 10⁻⁷ g/ml of 4-chloro-3-methyl phenol.

Except for the experiments in which the calcium concentration was increased before the addition of any other drug no significant changes in heart rate were observed in these studies. When calcium was added first there was usually an increase of about 25 per cent with the initial increase of calcium of one-fourth normal. No further increases were observed with greater amounts of calcium.

Discussion

For many years it was assumed that curare had no cardiac effects.[9][10] The absence of clinical or experimental evidence to the contrary attested to the validity of this assumption. In 1965, based on clinical observations of patients in whom d-tubocurarine had re-versed various ventricular arrhythmias, including fibrillation, and upon similar results obtained in dogs,[11] Dowdy and her co-workers studied the effects of d-tubocurarine in the isolated perfused rabbit heart. They observed a quinidine-like action of d-tubocurarine.[3] These authors did point out at the time that the d-curarine did not correct atrial fibrillation. In a subsequent study[4] it was reported that d-tubocurarine had a depressant action on the contractile tension of isolated left atria of rabbits which could be reversed by high concentrations of calcium. The results of the present study, however, indicate that d-tubocurarine has no depressant effect on atrial muscle except at very high concentrations (10⁻⁵ M), but that the vehicles in most commercial preparations contain substances which do depress the contractile tension of atrial tissue. In addition, this depression caused by the solvent is reversible by high calcium concentrations. The minimum concentration used both in vivo and in vitro[3][4] in the earlier studies (10⁻⁴ M), if used in the commercial injectable form (not specified in methods), would be accompanied by 8×10^{-5} M benzyl alcohol or 7.7×10^{-6} M 4-chloro-3-methyl phenol. We have observed that these substances at these concentrations can account for the depressant results obtained.

634   O. CARRIER, JR. AND J. C. MURPHY

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ACUTE DIURETIC RESPONSE OF GUANETHIDINE AND RESERPINE[1]

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at

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Running Title: Reserpine, Guanethidine Diuresis

FOOTNOTE

ABSTRACT

MURPHY, C. JAMES, JAMES B. JUSTICE, AND OLIVER CARRIER, JR: Acute diuretic response of guanethidine and reserpine.

Reserpine and guanethidine have been reported to cause a loss of rabbit aortic tissue electrolytes, however, no excretion routes for these have been defined. In the present study, blood and urine electrolyte changes were studied in dogs immediately after administration of 0.5 mg/kg I.V. reserpine, 15 mg/kg guanethidine or 5 ml of Serpasil placebo to see if tissue electrolyte loss was reflected in these fluids. Both reserpine and guanethidine produced an increase in urine volume and electrolytes. After reserpine blood sodium increased 10 mEq/l of plasma (7%) initially then dropped back to control levels. Other blood electrolytes were unchanged. Guanethidine did not effect any blood electrolyte substantially. The diuretic and saluretic response produced by these drugs lasted only during the first two hours after administration. It is concluded that the electrolyte losses observed in these studies can account for the major amount of vascular tissue sodium and potassium lost after reserpine administration, and for a part of the calcium loss.

INTRODUCTION

Reserpine (Serpasil) causes a significant loss of vascular
tissue calcium, sodium and potassium (1,2,3). These latter authors
(3) also obtained evidence that a great part of the calcium loss
appeared in the feces during the first two hours after administration
of the reserpine, however, no route for the sodium or potassium loss
has as yet been shown. It is thus of some interest to study the
effects of reserpine on urinary electrolyte excretion in order to
find the possible route of sodium and potassium loss caused by the
drug. Previous reports are not in agreement. Moyer, et al. (6)
reported that there was no change in electrolyte loss or urine out-
put following reserpine administration to dogs, while DeFelice (5)
reported a slight increase in urine output and sodium loss from
hydrated dogs given reserpine. It is known that the long term re-
sponse to reserpine is a decreased urine output thought to be
mediated by an increase in anti-diuretic hormone secretion (4).
However, no report of the short term effects of reserpine has been
made. Based on the fact that the electrolyte losses caused by
reserpine appear to peak during the first two hours (3) it is
possible that during this same time period changes in electrolyte
and water excretion may occur which could account for the vascular
tissue electrolyte losses.

The subject of the present report is the results obtained in
a study of the electrolyte and water excretion which occurs in dogs

upon acute intravenous administration of reserpine. It is shown that
during the first 90 minutes there is an increase in urine output and
urinary electrolyte excretion.

The antihypertensive agent, guanethidine (Ismelin) appears to
have many effects similar to those of reserpine though they are
thought to be mediated by different mechanisms. Both drugs lower
blood pressure, both lower catecholamine content of sympathetic nerve
ends, and both effect aortic tissue calcium (7,8,9). There are
differences however. Reserpine is lipid soluble; guanethidine is
water soluble. Guanethidine does not enter the central nervous system
as reserpine does. Both drugs have a long term anti-diuretic action.
Because of these similarities and differences it was thought to be
of value to use guanethidine in these studies as well as reserpine.

METHODS

Seventeen female mongrel dogs weighing 12-20 kg were used in
this study. The animals were anesthetized with 30 mg/kg sodium
pentobarbital. One femoral artery was cannulated from which blood
pressure was measured with a Statham pressure transducer and recorded
on an E&M Physiograph recorder. One femoral vein was cannulated for
injections and blood sampling. Both ureters were cannulated distal
to the bladder with PE-50 polyethylene tubing. A 45 minute equili-
bration period was allowed following the surgical procedures. After
equilibration three urine samples (10 minute volume) and three 5 ml
blood samples were taken. The average of these was taken as control.
All of the animals were injected intravenously with 15 mg/kg guanethi-

dine (Ismelin-CIBA), 0.5 mg/kg reserpine (Serpasil-CIBA) or 5 ml of Serpasil placebo. Five animals were pretreated with a total of 0.5 mg/kg reserpine over a two day period (0.35 mg/kg the first day and 0.15 mg/kg the second day) preceding the acute procedures. After administration of the drugs, blood and urine samples were taken as shown on the respective tables.

Serum and urine samples were analyzed for sodium and potassium content on an Instrumentation Laboratories flame photometer and calcium was determined with a Perkin-Elmer model 303 atomic abosrption spectrophotometer.

RESULTS

Effects of acute reserpine on urinary output of water and electrolytes. As shown in Table 1, 0.5 mg/kg reserpine caused both increased water and electrolyte losses during the first 10 minutes. The response from one dog to another was extremely variable but electrolyte excretion did peak in 30-45 minutes in all the animals. After the peak was reached, the excretion rate remained high for 20-40 minutes and then dropped off sharply. There was no significant change in blood pressure during the experimental period.

The average urinary electrolyte concentration in microequivalents per minute and urine volume in ml/min from 7 dogs is shown in Fig. 1. The shape of the calcium and sodium excretion rate curves are very similar to the urine output curve. There was, however, no apparent correlation between these and the potassium excretion rate.

Effects of Serpasil placebo on electrolyte and urine excretion. The responses by two animals after an injection of Serpasil placebo,

CIBA's vehicle for reserpine, are shown in Table 2. There was a slight increase in electrolyte excretion during the experimental period, and no significant change in urine output.

Effects of guanethidine on electrolyte and urine excretion. The animals given guanethidine (15 mg/kg) showed an increased urine output and electrolyte loss. Sodium and potassium losses were very similar to urine output. There was an immediate rise after drug administration in both electrolyte excretion and urine output which peaked in 20-30 minutes. Blood pressure rose about 50 to 70 mmHg and the rate and the duration of the rise resembled the urine and electrolyte curves (Table 3). Calcium excretion consistantly decreased after guanethidine administration but followed no set pattern and is not shown.

Effects of reserpine and guanethidine on electrolyte and urinary excretion in animals pretreat with 0.5 mg/kg reserpine. The blood pressure of the animals pretreated with reserpine for two days prior to the acute experiments was significantly lower than control animals. There was no apparent difference in urine output, and only in one animal was the sodium excretion rate significantly different. There was, however, a very obvious high excretion rate for potassium in four of the animals, a finding worthy of further investigation. There was a variable change in electrolyte loss after 0.5 mg/kg reserpine was administered acutely to animals which had been pretreated with 0.5 mg/kg reserpine over a two day period (Table 4). After 15 mg/kg guanethidine there appeared a great variability in sodium and potassium excretion, and no significant change in urine output during the experimental period. There was, however,

a decrease in blood pressure. One dog (Table 5) had a decrease in electrolyte excretion 20 minutes after guanethidine. A comparison of the maximum changes in electrolytes, blood pressure and urine output which occurred under all treatments used in these experiments is presented in Table 6.

Effects of guanethidine and reserpine on serum electrolytes. Guanethidine produced no detectable change in serum electrolytes. Reserpine caused a 10% rise in serum sodium at 20 minutes which was followed by a decrease of the same magnitude in sodium after 90 minutes. No detectable change in serum calcium or potassium concentration occurred (Fig. 2).

DISCUSSION

In the present study both reserpine and guanethidine produced an initial short term diuretic effect which preceded their well known anti-diuretic effect. Urine sodium, potassium and calcium excretion increased after reserpine. The curves describing these losses were very similar for sodium, calcium and urine. Potassium loss, which was much less then the sodium loss, followed a different time course which probably reflects the difference in renal handling of potassium from either sodium or calcium. The concentrations of sodium and calcium in the urine during the time of observation did not vary significantly. We are thus probably seeing simply the result of an increase sodium and calcium load presented to the kidney. This increased load being reflected by the slight rise in blood sodium content after reserpine. The sodium loss probably caused the concomitant water loss by osmosis. The potassium loss which is

much smaller is probably due to the high sodium in the urine in
the distal region of the tubules stimulating the sodium-potassium
exchange mechanism. These data suggest that the reserpine
mediated electrolyte loss from vascular tissue previously reported
(1,2,3) occurs in the following sequence: sodium, potassium and
calcium leave the vascular tissue to enter the blood stream to be
excreted. Excretion of calcium then occurs principally through the
intestine, while the principle loss of sodium and potassium occur
in the urine. These events appear to occur only during the first
few hours after one dose of reserpine.

The response produced by guanethidine may be related to the
increased blood pressure which occurs during the first hour or so
after its administration because the time course of urine and electro-
lyte losses were very similar to the blood pressure change. Reserpine
caused no such increase in blood pressure and its effects on calcium
excretion were qualitatively different from that of guanethidine.
Since neither guanethidine nor reserpine produced definite trends
in electrolyte excretion in reserpine pretreated dogs it would
appear that at least part of the observed changes which occurred
after administration of both drugs was related in some manner to
catecholamine release by sympathetic nerve ends. However, pretreatment
with reserpine 48 hours before an acute experiment would also pre-
vent further electrolyte losses during the acute experiment.

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LEGENDS

Fig. 1:   Mean urine and electrolyte excretion rates for 120 minutes after acute administration of 0.5 mg/kg reserpine to seven dogs. Ordinate: urine output in ml/min, sodium (————), potassium (— ·· — —) and calcium (— · — · —) excretion rates in mEq/min. Abscissa: time in minutes.

Fig. 2:   Plasma sodium (— — —), potassium (— - - —) and calcium (— · — · —) concentrations for 100 minutes after administration of 0.5 mg/kg reserpine to seven dogs. Ordinate: plasma electrolyte concentrations in mEq/l. Abscissa: time in minutes.
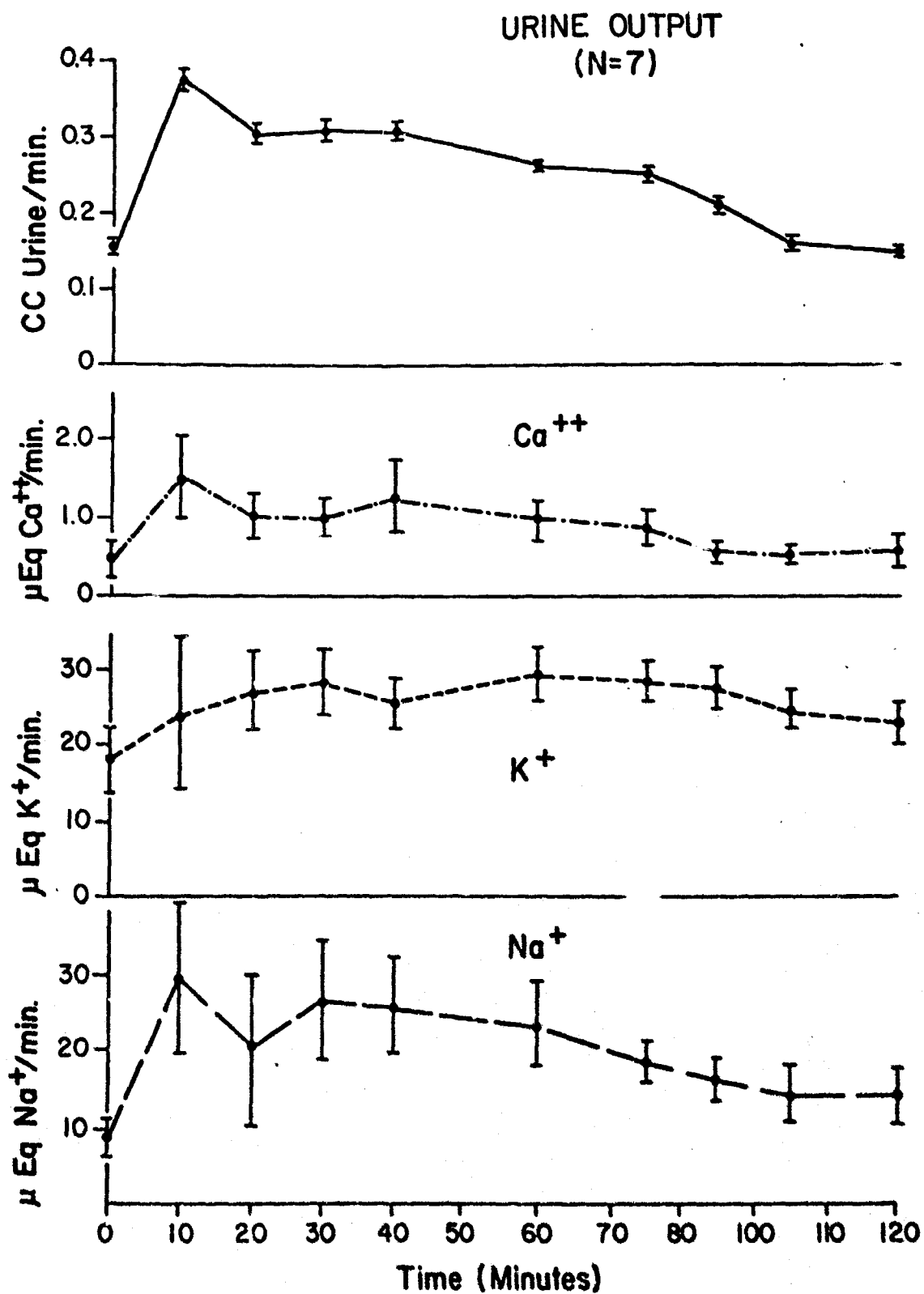
FIGURE 1
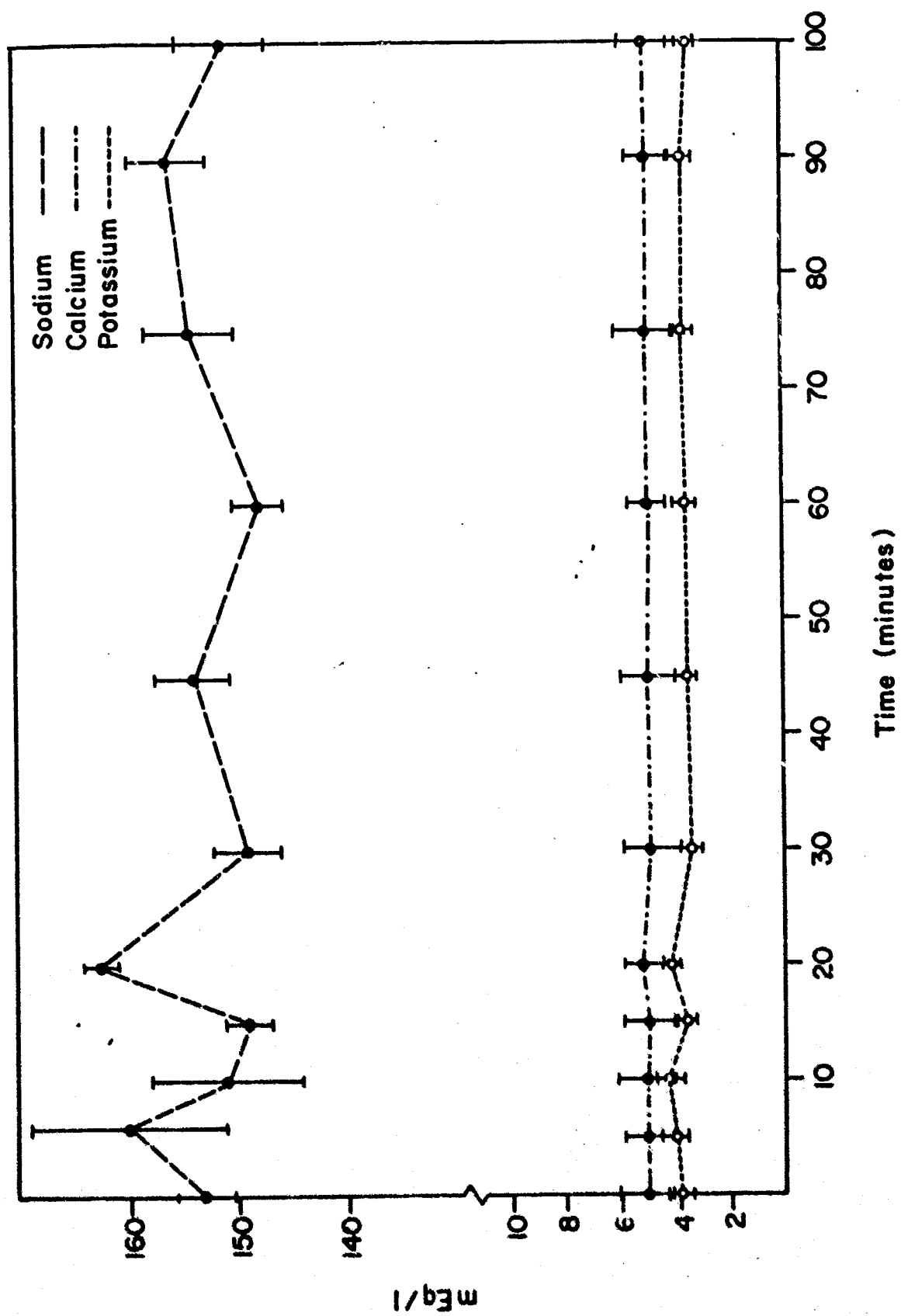
FIGURE 2

Table 1
ELECTROLYTE AND URINE EXCRETION RATES AFTER (0.5 mg/kg) RESERPINE IN 4 DOGS*

Time After Reserpine	0	5	10	15	20	25	30	35	40	45	60	75	90	105	120
Dog 1 (18 kg)															
μEq Na$^+$/min	6.2		23.2		25.1		51.9		42	38	35.6	29.7	22.9	13.8	8.5
μEq K$^+$/min	13.7		32.6		24.6		37.5		29	22.3	24.8	26.2	20.9	12.5	7.4
μEq Ca/min	0.28		0.81		0.49		1.05		0.94	0.94	1.08	1.08	0.84	0.53	0.28
ml urine/10 min	0.8		1.8		1.4		3.0		2.5	2.5	2.7	2.7	2.4	1.5	0.8
Dog 2 (15 kg)															
μEq Na$^+$/min	3.6	3.5	7.4	10.6	10.2	17	22.8	43	42.2	37.2	27.4	14.1	7.1	3.5	3.9
μEq K$^+$/min	3.8	4.2	5.6	9.4	7.8	11.4	13.2	23.6	23.7	23.5	18.5	23.2	30.3	23.7	27
μEq Ca/min	0.27	0.25	0.46	0.96	0.92	1.29	1.8	3.7	3.5	2.3	2.0	1.2	0.58	0.34	0.34
ml urine/10 min	0.6	0.6	1.0	2.0	2.0	3.0	4.0	6.2	6.4	4.8	3.7	2.9	3.4	2.6	2.6
Dog 3 (15 kg)															
μEq Na$^+$/min	3.8	5.4	6.3	4.3	6.4	12.5	7.2	10.9	15.3	22	15.6	26.7	24.6	24.5	16.5
μEq K$^+$/min	5.8	9.0	10	11.4	12.6	16.5	15	15.1	15.5	27	24.5	29	28.5	27	25.6
μEq Ca/min	0.19	0.3	0.3	0.42	0.36	0.49	0.4	0.48	0.46	0.7	0.63	0.54	0.63	0.53	0.39
ml urine/10 min	0.7	1.0	1.0	1.0	1.2	1.4	1.0	1.0	1.0	2.0	1.8	1.8	1.6	1.5	1.2
Dog 4 (16 kg)															
μEq Na$^+$/min	51.3	177	173	125.1	137.3	105	83.5	77.5	54.5	41.4	24.4	15.9	9.8	5.6	2.8
μEq K$^+$/min	14.3	26	24	22.9	32	29.4	25.5	25.5	20.9	19.5	16.5	13.3	15.1	9.9	10.2
μEq Ca/min	1.9	4.1	4.0	3.0	2.7	2.5	2.0	2.0	1.5	1.3	0.9	0.9	0.5	0.4	0.2
ml urine/10 min	3.0	10.0	10.0	7.4	7.8	7.0	5.0	5.0	3.6	3.2	2.2	2.3	2.1	1.8	1.1

0 = Control

* = Mean blood pressure in all dogs (7) was 143.5± 3.7. There was no significant change during the experimental period.

Table 2

ELECTROLYTE AND URINE EXCRETION RATES AND BLOOD PRESSURE RESPONSES AFTER SERPASIL PLACEBO (5cc).

Time After Drug	0	10	20	30	40	50	60	70	80	90	100
Dog 1 (12kg)											
μ Eq Na^+/min	2.95	5.25	6.1	7.80	8.77		6.95		8.58		6.95
μ Eq K^+/min	5.81	16.8	19.8	21.36	24.31		20.1		27.43		19
ml urine/10 min	1.0	1.0	1.1	1.2	1.3		1.05		1.3		1.0
Blood pressure (mmHg)	150	150	150	150	150		150		150		150
Dog 2 (14kg)											
μ Eq Na^+/min	42.7	68.7	32.6	55.9			66.5		48.8		18.6
μ Eq K^+/min	21.28	32.7	16.5		20.4		21.1		32.4		12.0
ml urine/10 min	4.15	3.0	1.3		2.25		2.52		2.05		1.0
Blood pressure (mmHg)	150	140	130	130	130	130	130	120	130	100	100

Table 3

ELECTROLYTE AND URINE EXCRETION RATES AND BLOOD PRESSURE RESPONSES AFTER GUANETHIDINE (15mg/kg).

Time After Drug	0	10	20	30	40	50	60	70	80	90	100
Dog 1 (20kg)											
μ Eq Na$^+$/min	31.65	560	393.9	136.7	145.2	141.75	145.20	86.03	55.20	34.30	
μ Eq K$^+$/min	27.19	95.20	89.70	52	45.6	46.2	48.8	31.45	27.2	22.4	
ml urine/10 min	2.53	56	39	26	24	21	22	18.5	15	14	
Blood pressure (mmHg)	130	240	175	150		130	130	140	150	160	
Dog 2 (14kg)											
μ Eq Na$^+$/min	3.60	49.84	111.78	113.96	106.94	90	68.63	82.69	82.08	70.74	66.51
μ Eq K$^+$/min	10.02	29.12	37.40	29.92	30.45	28.2	22.5	27.54	30.24	27.73	27.26
ml urine/10 min	.46	2.8	8.5	8.8	7.25	6.0	4.5	5.4	5.4	4.7	4.7
Blood pressure (mmHg)	150	220	200	200	200	200	200	180	190	175	175
Dog 3 (14kg)											
μ Eq Na$^+$/min	3.57	57.81	69.96	54.51	71.05	78.60	63.25	58.31	61.49	60.60	67.32
μ Eq K$^+$/min	21.27	50.76	64.02	42.09	47.7	42.0	36.3	46.06	36.98	34.80	35.64
ml urine/10 min	1.2	4.7	6.6	6.9	7.0	6.0	5.5	4.9	4.3	4.0	4.4
Blood pressure (mmHg)	130	175	175	185	175	185	185	175	175	160	160

Table 4

ELECTROLYTE AND URINE EXCRETION RATES AFTER RESERPINE (0.5 mg/kg) IN 3 ANIMALS PRETREATED WITH RESERPINE (0.5 mg/kg).

Time After Reserpine	0	10	20	30	40	50	55	60	70	75	90
Dog 1 (14kg)											
μEq Na$^+$/min	12.0		17.5	21.5	16		23.5		20		
μEq K$^+$/min	33		44	39	38		45		52		
ml urine/10 min	0.5		0.5	1.16	1.10		0.73		0.6		
Blood Pressure (mmHg)	90		90	90	90		90		90		
Dog 2 (16kg)											
μEq Na$^+$/min	3.5	3.0	3.5	3.5	4.0	4.0		4.5		6.0	5.5
μEq K$^+$/min	73	80	89	95	96	98		106		87	71
ml urine/10 min	1.41	1.52	1.41	1.38	1.36	1.53		0.98		0.85	0.8
Blood Pressure (mmHg)	85	87	87	87	87	85		85		85	85
Dog 3 (20kg)											
μEq Na$^+$/min	251.5	251.5	251.5	251.5	246.5	234		220.5		206	202
μEq K$^+$/min	201	210	197	221	237	251		251		251	251
ml urine/10 min	1.35	1.32	1.4	1.39	1.35	1.53		1.53		1.6	1.6
Blood Pressure (mmHg)	88	88	88	89	89	90		88		87	89
Dog 4 (14kg)											
μEq Na$^+$/min	36	22.5	25.5	27.5	25.5						
μEq K$^+$/min	251	251	251	251	251						
ml urine/10 min	0.4	0.4	0.3	0.45	0.4						
Blood Pressure (mmHg)	95	94	94	95	95						

Table 5

ELECTROLYTE AND URINE EXCRETING RATES AND BLOOD PRESSURE RESPONSES AFTER GUANETHIDINE (15mg/kg) IN ANIMALS PRETREATED

WITH RESERPINE (0.5mg/kg).

Time After Guanethidine	0	10	20	30	40	50	60	70	80	90	100
				Dog 1 (14kg)							
μ Eq Na$^+$/min	1.20	6.03	1.56			6.4	7.39			1.37	
μ Eq K$^+$/min	3.23	8.7	1.84			4.5	6.97			.61	
cc urine/10 min	0.70	.9	0.4			0.4	.85			.15	
Blood Pressure (mmHg)	60	50	50			50	50			50	
				Dog 2 (14kg)							
μ Eq Na$^+$/min	21.59	28.96	10.71	6.82	5.42	5.10	6.1	5.1	5.39	5.23	
μ Eq K$^+$/min	30.63	24	15.54	10.12	4.94	4.76	4.0	3.4	5.06	6.96	
cc urine/10 min	2.6	3.2	2.1	2.2	1.9	1.7	2.0	1.7	2.2	2.4	
Blood Pressure (mmHg)	125	85	90	95	100	100	100	100	110	120	

Table 6

A COMPARISON OF THE MAXIMUM CHANGE IN ELECTROLYTES, URINE OUTPUT AND BLOOD PRESSURE OBSERVED AFTER THE VARIOUS DRUG TREATMENTS

Control	0.5mg/kg Reserpine(I.V)	15mg/kg Guanethidine(I.V)[b]	Placebo(I.V)	0.5mg/kg Reserpine(I.V) (Animals Pretreated 48 Hours With Reserpine)	15mg/kg Guanethidine(I.V) (Animals Pretreated 48 Hours With Reserpine)
	Change from control			Change from control	
Calcium 1.0[a]	5.45 ± 2.1*	0.95 ± 0.1	1.0 ± 0.0	1.0 ± 0.0	0.80 ± 0.07
Potassium 1.0	4.49 ± 1.3	3.41 ± 0.2*	3.13 ± 1.6	1.29 ± 0.1*	1.74 ± 1.0
Sodium 1.0	7.64 ± 1.7*	23.85 ± 4.1*	2.29 ± 0.7*	1.35 ± 0.3	3.75 ± 2.4
Urine Output 1.0	5.07 ± 1.8*	15.76 ± 5.1*	1.01 ± 0.3	1.43 ± 0.3	1.26 ± 0.03*
Blood Pressure 1.0	1.0 ± 0	1.58 ± 0.1*	0.99 ± 0.02	1.0 ± 0	0.90 ± 0.1

a Mean of controls for each experiment set equal one in order to compare the data

b Values given are times the control value for the particular group. See other Tables and Figures for actual data.

* Significantly different from control.

Supersensitivity in vascular smooth muscle initiated by the cold [1]

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Running Title: Cold supersensitivity

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FOOTNOTE

ABSTRACT

MURPHY, JAMES C., AND OLIVER CARRIER, JR. <u>Supersensitivity in</u>
<u>vascular smooth muscle initiated by the cold</u>. Am. J. Physiol.
The isolated branch of the dog femoral artery was found to be super-
sensitive to norepinephrine after 24 hours refrigeration in normal
Ringer's. The rabbit aorta could only be made supersensitive in 24
hours if twice normal calcium (4.8 mM) was added to the Ringer's
solution prior to refrigeration. The potentiation observed in the
rabbit aorta was greater at lower doses of norepinephrine, while
potentiation of the femoral responses was greater at the higher
doses of norepinephrine. Angiotensin and vasopressin responses
were depressed after cold storage. However, vasopressin was more
responsive after rapid cooling and reheating. Cold storage in the
presence of 1×10^{-5} g/ml norepinephrine had no effect on the re-
sults. Electrolyte changes were studied and the data suggest that
an increased availability of calcium to the contractile mechanism
after cold storage may be the cause of supersensitivity.

cold storage; temperature effects; vascular tone; vascular response;
norepinephrine; angiotensin, vasopressin; sodium; potassium; calcium;
supersensitivity

Changes in temperature can result in very important changes in cardiovascular function. This becomes especially notable in the vasculature. The very well known and vital responses of skin and other regional blood vessels to changes in the external environment temperature is only one example of the role temperature changes may play. Several studies have indicated that most vessels are less responsive to stimuli at low temperature, and that they constrict in response to the cold. Folkow (4) found that temperatures below 37°C produced acute vasoconstriction in both man and animals. This has also been shown by several other groups (7,8,9,12). The fact that this acute response to cold can be prevented in situ by alpha-adrenergic block' g drugs or sympathectomy indicates an involvement of the autonomic nervous system.

It is well known that temperature changes can affect the results of studies of isolated vascular tissues. When drug responses are determined using isolated muscle preparations, the organ bath temperature must be maintained constant in order to obtain reproducible results. In a recent review Somlyo and Somlyo (14) stated that drug responses vary with temperature. They point out that among different types of blood vessels this temperature dependence is variable, but in most, if not all, instances there is a minimum temperature below which the smooth muscle no longer responds at all to drugs or other stimuli.

Though there are several studies in the literature on the effects of temperature, most are incidental to some other aspects of the

studies. There are none which have produced evidence on the mechanism of action of changes induced by temperature variation. It was thus of some interest to study the influence of temperature change on blood vessels which may be of importance in contributing to the peripheral resistance of the cardiovascular system, and to attempt to elucidate the mechanism involved. The subject of the present report is the result of such a study.

METHODS

Isolated Perfused Blood Vessels

Blood vessels. The vessels used were lateral branches of the femoral artery of the dog and were approximately 2 cm in length and 0.5 mm inside diameter. They were obtained from mongrel dogs weighing 10-15 kg. The animals were anesthetized with 30 mg/kg sodium pentobarbital, given 2 mg/kg heparin i.v., and bled. Immediately after the death of the dog the vessels were cannulated in situ with two twenty gauge needles which had been blunted, buffed, and standardized. The needles were tied in the vessels securely with their tips 1 cm apart. After removal from the animal, the vessels were placed in oxygenated, warm Tyrode's solution (37°C) until used.

Apparatus. Each vessel used was mounted in one of two identical chambers containing Tyrode's solution and perfused under a constant pressure of 100 mmHg from a reservoir placed at the height necessary to maintain the desired pressure. A diagramtic drawing of apparatus is shown in Figure 1 (for further details of the method see Carrier and Holland (2)). Chamber temperature was regulated by changing

the temperature of water passing from the Lauda constant temperature water bath through the outer jacket of the perfusion apparatus. The chamber medium, as well as the perfusate, was oxygenated with a 95% oxygen and a 5% carbon dioxide gas mixture. The pH of the bath and perfusate was maintained at pH 7.4 ± .05. The perfusate passed through a series of coils located in the outer jacket of the chamber before reaching the vessel so that the perfusate and bath solutions were at the same temperature. Pressures were measured with an E&M transducer and recorded on an E&M Physiograph recorder. Flow through the vessels was determined by an E&M quartz crystal drop counter and recorded on the Physiograph recorder.

Procedure. Both fresh and refrigerated vessels were studied by this technique. The fresh vessels used as controls were mounted in the chamber within one hour after removal from the animal and then perfused at 37°C for one hour before the experiment was started. The refrigerated vessels were stored in Ringer's solution at 6°C for various periods of time. After this refrigeration period, they were allowed to warm to room temperature while being oxygenated for 60 minutes before being placed in the chamber. After being placed in the perfusion chamber these vessels were then treated in the same manner as the fresh vessels. Test drugs were injected via a three way stopcock in the perfusion system just ahead of the vessel. Concentrations shown in Figures and Tables are total free base injected. (One has to assume this is the approximate concentration the vessel is subjected to; actual concentrations at receptor is dependent upon flow rate, vessel lumen, etc.). Temperature was decreased

or increased in increments of 3 degrees centigrade and dose-response curves were obtained at each temperature.

After the initial equilibration period, some of the fresh vessels were rapidly cooled to 27°C, then returned to 37°C and retested to determine if any changes took place during the rapid cooling. Temperature was then raised and the response to drugs at higher temperatures was studied. An alpha adrenergic blocking agent was used to determine if any change in receptor affinity occurred during either rapid cooling or storage. The dose-response relationships were obtained by determining the decrease or increase in flow (drops/min) at constant pressure. After each test dose of a particular drug, the vessel was allowed to return to control flow before a second dose was applied. There was as a result a minimum of twenty minutes between each dose. Drugs were injected in 0.1 ml volumes. All drugs were prepared for use from concentrated stock solutions just prior to their use. Stock solutions were kept in the refrigerator, and all solutions were made with deionized, glass distilled water.

Rabbit Aortic Strip Preparation

Albino rabbits of both sexes weighing 2 kg were stunned by a blow on the back of the head and bled via the carotid arteries. The thoracic aorta was removed and placed in oxygenated Ringer's solution. The excess fat and connective tissue were then cleaned from the vessels. Spiral helical strips 2 to 3 mm wide and about 20-30 mm in length were then prepared. Four strips were cut from each aorta and mounted vertically in 100 ml organ chambers.

The strips were tied at one end to a glass rod in the lower part of the chamber and the other end tied to a Grass FT-03 strain gauge.

The chambers were filled with Ringer's solution. The temperature and pH were monitored and maintained at 37°C and pH 7.4 ± 0.5. A gas mixture of 95% O_2, 5% CO_2 was bubbled through the solution throughout all procedures. Refrigerated vessels were maintained in normal Ringer's solution, zero-calcium Ringer's solution, or twice-normal-calcium Ringer's solution at 6°C for 1 to 4 days. No gas was added to this medium during the refrigeration period. After the refrigeration period these vessels were washed three times in normal Ringer's solution, warmed to 37°C, then treated in the same manner as the non-refrigerated vessels. After the strips were mounted in the organ chambers, 1.5 g tension was applied one hour prior to testing with the drugs and maintained throughout the experiment. The vessels refrigerated in zero-calcium Ringer's solution were tested in low calcium Ringer's solution.

The aortic strips, after being exposed to various temperature conditions (See Table 2), were subjected to various doses of norepinephrine or angiotensin and isometric contractions were recorded by means of the strain gauges and a Grass model 7-polygraph recorder. The dose-response relationships for norepinephrine were obtained by measuring the responses to cumulative doses of norepinephrine. The volume of drug solution added was 0.7 ml, and at least 30 minutes were allowed between successive experiments with the same strip. In a few experiments, lanthanum chloride or manganese chloride was used to test the extracellular calcium dependence of the observed contractile response.

All drug solutions were prepared from concentrated stock solutions immediately before an experiment. All solutions were made with deionized, glass-distilled water. Drug concentrations shown in results are final bath concentrations to which the tissues were exposed.

Calcium[45] studies. In these studies, rabbit aortic strips were
allowed to equilibrate for one hour in normal Ringer's solution. At
the end of this period 20 microliters of a stock solution of $Ca^{45}Cl_2$
(specific activity; 0.2 millicuries/ml) was added to the bath. The
tissue was exposed to the isotope for 30 minutes, after which it was
washed 3 times with non-radioactive Ringer's solution to remove excess
calcium[45] from the extracellular spaces, blotted dry with filter
paper, weighed, and digested. The digestion was done by placing the
tissue in a volume of NCS solubilizer (Amersham/Searle) six times
the weight of the tissue (i.e. 6 ml/mg) overnight at 50^oC. Follow-
ing the digestion a volume of glacial acetic acid equal to 1/30th of
the volume of solubilizer was added to the tissue mixture. This final
solution was placed in a scintillation vial and brought to a total
volume of 20 ml with a 6% (volume/volume) solution of PPO-POPOP in
toluene. The counting was done using a Nuclear-Chicago Unilux II
Liquid Scintillation counter.

Tissue electrolytes. Tissues used for electrolyte analysis
were taken either directly from the animal or after various treat-
ments and placed in polypropylene test tubes (50 ml). They were
dried at 100^oC overnight and weighed. The dry tissue was digested
in concentrated nitric acid. After digestion was complete the nitric
acid solution was diluted to the proper concentration with lanthanum
chloride for calcium determination on a Perkin-Elmer Model 303 Atomic
Absorption Spectrophotometer, or with lithium chloride for determination
of sodium and potassium on an Instrumentation Laboratories Flame Photometer.

<u>Drugs and solutions used.</u>

<u>Drugs</u>: Acetylcholine bromide, angiotensin (Hypertensin), norepinephrine (Levophed), phentolamine (Regitine), and Lysine-vasopressin (Sandoz).

<u>Tyrode's solution</u>: (mM) NaCl, 136.8; KCl, 2.65; $CaCl_2$, 1.8; $MgCl_2$, 1.05; NaH_2PO_4, 0.36; $NaHCO_3$, 12.0 and dextrose[6], brought to 1 liter with deionized, glass-distilled water.

<u>Ringer's solution</u>: (mM) NaCl, 154; KCl, 5.4; $CaCl_2$, 1.2; $NaHCO_3$, 6.0; $MgCl_2$, 1.0; dextrose, 11.0 brought to 1 liter with deionized, glass-distilled water.

RESULTS

<u>Effects of temperature on the isolated perfused vessels.</u> The results obtained when the temperature of the isolated perfused vessels was lowered or raised are shown in Figure 2. The vessels were either cooled or heated after equilibration at 37°C, but never subjected to both heat and cold. When the temperature was lowered to 25°C flow decreased to 83% of control. When the temperature was increased to 45°C the flow increased to 117% of control. Flow in the vessels which had been refrigerated overnight at 6°C decreased to 76% of control when cooled to 25°C. When the refrigerated vessels were warmed they responded very different from the fresh vessels. In these, as soon as the bath temperature reached 38°C, flow increased 10% from control and remained at this level until the temperature reached 40°C. From this point on, as the temperature was increased, the flow decreased until at 45°C it was 60% of control.

Both refrigerated and fresh vessels had an increase in flow at
37°C after being rapidly cooled to 27°C (Figure 3). Flow, however,
decreased in both the refrigerated and fresh vessels at temperatures
above 41°C after an initial increase of 20% at 39°C.

As illustrated in Figure 4, the response of fresh vessels to
different concentrations of norepinephrine at 37°C was greater than
that at 27°C. Norepinephrine responses were determined at several
temperatures between 27 and 37°C. There was a progressive decrease
in response as the temperature was decreased. Refrigerated vessels
responded qualitatively the same as normal vessels but were much
more responsive to higher doses of norepinephrine than were the fresh
vessels. The resistance in fresh vessels maintained at 37°C in-
creased 4% when subjected to 8×10^{-6} gm/ml of norepinephrine, while
at 27°C this same concentration of norepinephrine caused a decrease
in resistance to 83% of control, indicating that flow actually in-
creased at this low temperature. The resistance of the refrigerated
vessels at 37°C increased 8% with 8×10^{-6} g/ml norepinephrine while
there was relatively no response at 27°C. At a concentration of
1×10^{-4} gm/ml norepinephrine the refrigerated vessels' resistance
increased 180% at 37°C while the increase was only 48% at 27°C.
There were no significant alterations in drug response of either
the fresh or refrigerated vessels as the temperature was increased
from 37°C to 45°C. However, the refrigerated vessels were more re-
sponsive than fresh vessels at all concentrations above 8×10^{-6}

gm/ml of norepinephrine at the higher temperatures. In 24 experiments the effects of 10^{-7} gm/ml phentolamine were studied. At this concentration norepinephrine responses were readily blocked while there were no significant effects on the vascular responses to changes in temperature.

The isolated perfused vessels did not respond to acetylcholine or angiotensin under any conditions. As illustrated in Table 1 the vessels responded to vasopressin at temperatures between 37°C and 45°C but were not responsive below 32°C. The vessels appeared to be more responsive to vasopressin if they were rapidly cooled, first to 27°C then rewarmed to 37°C, prior to administration of vasopressin (Table 1). The vessels did not respond to vasopressin after 24 hours refrigeration at 6°C as illustrated in Table 1. Phentolamine (10^{-7} gm/ml) had no effect on the vascular responses to vasopressin.

Effects of refrigeration on drug responses of aortic strips. In Table 2 the various conditions under which the rabbit aortas were studied and how these conditions altered contractility are shown. These data indicate essentially no difference in response of rabbit aortas to norepinephrine after 24 or 48 hours refrigeration at 6°C in normal Ringer's solution or Ringer's solution containing 1×10^{-5} gm/ml of norepinephrine. The norepinephrine was added to prevent catecholamine depletion during refrigeration. After 4 days refrigeration in normal Ringer's solution, however, the vessels were more responsive at 1×10^{-8} gm/ml norepinephrine but not at higher concentrations. After refrigeration at 6°C in twice-normal

calcium Ringer's solution for 24 or 96 hours, the vessels were more responsive at all doses of norepinephrine when tested at 37°C. At 1×10^{-8} gm/ml there was a 14-fold increase in response. This increased response after refrigeration in a high calcium medium could be inhibited with 4×10^{-3}M LaCl$_3$ or 1×10^{-3}M MnCl$_2$.

Aortas, after refrigeration in zero calcium Ringer's solution, were responsive for a short period of time to norepinephrine when tested in a low calcium Ringer's solution. These responses were not as great as those of tissues refrigerated and tested in normal Ringer's solution. However, even though these tissues responded but once, the response was significantly greater than that of fresh vessels at the same concentration (10^{-8} gm/ml norepinephrine). These vessels would not respond to a 2nd or 3rd dose of 1×10^{-8} gm/ml of norepinephrine. Responses at all test doses were greatly reduced after the first test.

Aortas which had been refrigerated for 24 hours or 48 hours in normal Ringer's or Ringer's containing 1×10^{-5} gm/ml of norepinephrine, were less responsive to angiotensin than fresh vessels (Table 2).

Aortic electrolytes. In Table 3 the electrolyte content of aortic tissue under various conditions is shown. There was an increase in tissue sodium and a decrease in tissue potassium during refrigeration. This was unaffected by incubation in the presence of 1×10^{-5} gm/ml of norepinephrine or twice normal calcium in Ringer's solution. When the Ringer's solution contained no calcium, the potassium decrease was 50% greater. Vessels incubated 24 hours at 37°C had similar changes in their sodium and potassium contents.

Table 4 shows the calcium changes which occurred in the tissue. There was a significant increase in tissue calcium in vessels refrigerated 24 hours in normal Ringer's solution and an even greater increase in tissue refrigerated in Ringer's solution containing twice normal calcium. The tissue calcium was unchanged in vessels incubated at $37^{\circ}C$ in normal Ringer's. We were unable to measure any calcium in the tissue incubated 24 hours in zero calcium Ringer's solution.

The Ca^{45} uptake was decreased 10-fold in aortas refrigerated 24 hours in normal Ringer's solution. The vessels refrigerated in Ringer's solution containing twice normal calcium took up the same amount of Ca^{45} as did the fresh contr' vessels.

DISCUSSION

The present studies confirm what previous workers have proposed (5), that norepinephrine works in a much differ at manner than do other vasoconstrictors. We saw an increased response to norepinephrine after refrigeration, while the responses initiated by angiotensin and vasopressin were depressed. Phentolamine blocked the norepinephrine responses but had no effect on the responses produced by the other vasoconstrictors or the changes in myogenic tone seen with changes in temperature. In the present study it was observed that the isolated dog femoral was supersensitive to norepinephrine after 24 hours refrigeration in normal Ringer's. We observed as did Shibata (13), that the rabbit aorta does not show an increased response to norepinephrine after 24 hours refrigeration but does after prolonged re-

frigeration. The mechanism where: vascular tissues shows this in-
crease in sensitivity after refrigeration is probably related to some
change in the tissue ionic profile. However, the sodium and potassium
changes we observed could not be correlated with the sensitivity
changes. Sodium increased in the tissue and potassium was lost when
the vessels were incubated at 37°C while calcium levels remained un-
changed and tissue sensitivity decreased. In the cold we saw the same
effects on sodium and potassium. However, tissue calcium levels in-
creased as did tissue sensitivity.

Tissue electrolyte changes occurring in the cold or at room
temperature, nor supersensitivity after cold were altered by incubation
in the presence of norepinephrine. Norepinephrine was apparently
unable to protect the sodium pump since electrolyte changes were un-
altered. However, it probably helped to maintain tissue norepinephrine
levels, therefore preventing a lack of norepinephrine from playing a role
in the sensitivity change.

The change in sensitivity observed in these studies after refriger-
ation is probably not due to a generalized increase in membrane perme-
ability since it was only true for the norepinephrine responses and not
the other vasoconstrictor drugs. It was clearly not due to a simple
increase in tissue calcium since the increase in responsiveness in the
aorta which appeared after rewarming to 37°C was still present after
incubating for one to four hours at 37°C at which time the tissue
electrolyte content was back to control values. Also, there was a four-
fold increase in tissue calcium of rabbit aortas refrigerated in normal
Ringer's solution 24 hours but no change in sensitivity and these

vessels appeared to be less permeable to calcium[45]. It thus appears
that some change occurred during the cold storage of the vessels
which altered some facet of the smooth muscle cell's contractile re-
sponse mechanism. It appears from the present studies that after
refrigeration the cell may change to a state more suited to utilize
calcium similar to that which occurs after reserpine, for increased
responsiveness appeared even in vessels incubated and tested in zero-
calcium Ringer's solution. In a high calcium medium the aortic strips
were more responsive than either normal aortas or aortas refrigerated
for four days in normal Ringer's. However, the contractile responses
of the vessels could be blocked by addition of either lanthanum
chloride or manganese chloride to the bath indicating that extra-
cellul r calcium was still necessary for the supersensitivity due to
the cold.

The fact that vasopressin responses appeared to increase after
rapid cooling and rewarming suggests that responses produced by other
drugs may also be altered by cooling tissues to various temperatures
for different periods of time. Thus, at least some step in the con-
tractile process which common t a'' such responses must be sought.
The present studies suggest an increased utilizable calcium. It has
been postulated that the supersensitivity which develops after reser-
pine is due to an increase in receptor area of the effector muscle,
and that this change may be related to a calcium loss which occurs (3).
More recent work suggest that the tissue calcium loss, which is
apparently only transient at moderate doses of reserpine (1), may only

be coincidental with some intracellular event involving calcium's availability to the contractile apparatus (11). Lahrtz, et al (1967) have proposed that cold inhibits active transport of calcium out of the cell or into the endoplasmic reticulum while it may still passively enter the cell. This, in effect, could cause an increase in intracellular bound or ionized calcium or both which remains after the tissue is rewarmed accounting for the increased responsiveness.

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ACKNOWLEDGEMENT

Table 1. Perfusate flow of blood vessels in response to vasopressin at different temperatures (expressed as the fraction of control flow).

Fresh Vessels

Temperatures °C	Concentration of Vasopressin (I.U./ml)				n[c]
	1	3	5	7	
45	0.94±.05[b]	0.83±.10	0.79±.10	0.76±.11	6
41	1.03±.04	0.90±.05	0.80±.07	0.79±.07	8
37	0.95±.02	0.79±.06	0.70±.05	0.65±.05	8
32	0.95±.08	0.99±.05	0.97±.03	0.99±.05	3
27	1.04±.05	1.05±.07	1.00±.06	1.02±.05	3
Vessels cooled to 27°C then rewarmed 37°C					
37	0.99±.05	0.62±.11	0.46±.11	0.42±.13	6
41	1.03±.11	0.61±.19	0.35±.07	0.31±.12	6
45	1.02±.14	0.64±.30	0.55±.22	0.33±.13	6

Refrigerated Vessels[a]

Temperatures °C	Concentration of Vasopressin (I.U./ml)			
	1	3	5	7
45	1.14±.08	0.99±.04	1.02±.03	1.04±.02
41	0.98±.02	0.95±.03	0.94±.04	0.94±.03
37	0.99±.01	0.94±.03	0.94±.02	0.94±.03
32	*			
27	*			

* No response at any concentration of vasopressin below 37°C

a Vessels refrigerated at 6°C for 24 hours prior to experiment. Treated identically to controls (fresh) vessels upon rewarming to 37°C.

b Mean with standard error of the mean flow expressed as decimal fraction of control flow. A decrease in flow assumed to reflect a decrease in radius of the vessel.

c Number of experiments.

67.

TABLE 2. Responses of isolated rabbit aortic strips to norepinephrine and angiotensin after various treatments

Experimental Conditions [a]	Norepinephrine (g/ml)				Angiotensin	
	10^{-8}	10^{-7}	10^{-6}	N	5×10^{-8} g/ml	N^1
Control	0.06±.04[b]	1.42±.12	2.57±.21	16	2.08±0.10	5
24 hrs, 6°C, N-Ringer's	0.24±.08	1.25±.16	2.15±.18	12	1.35±0.16	12
48 hrs, 6°C, N-Ringer's	0.10±.04	1.75±.22	2.95±.31	4	1.50±0.22	5
24 hrs, 6°C, N-Ringer's 10^{-5} g/ml Norep.	0.19±.03	1.12±.09	2.0±.14	12	1.56±0.11	12
4 days, 6°C, N-Ringer's	0.45±.07	1.98±.14	2.68±.21	12		
4 days, 6°C, N-Ringer's 10^{-5} g/ml Norep.	0.47±.07	2.09±.14	2.68±.12	16		
24 hrs, 6°C, 2X Ca^{++} Ringer's	1.11[c]±.13	3.10±.19	4.25±.15	12		
4 days, 6°C, 2N Ca^{++} Ringer's	1.39±.09	3.12±.16	3.89±.19	24		

[a] Strips stored under listed conditions for given time prior to the experiment.

[b] Mean response in grams tension plus standard error of the mean.

[c] Responses after cold storage in high calcium Ringer's solution could be blocked by 4×10^{-3} M $LaCl_3$.

N and N^1 = number of strips tested.

TABLE 3.   Rabbit aortic tissue electrolyte content after various treatments

Conditions	N	Sodium Content mEq/kg dry tissue	Potassium mEq/kg dry tissue
Fresh[a]	6	299.93 ± 10.3[c]	131.80 ± 9.6
Fresh, Ringer's[b]	17	424.24 ± 23.5	111.18 ± 4.97
24 hrs at 6°C in Ringer's tested with 10^{-5} g/ml NE	10	465.91 ± 40.39	100.45 ± 7.52
24 hrs at 6°C in Ringer's tested with 10^{-5} g/ml NE	8	379.19 ± 27.00	79.52 ± 10.03
24 hrs at 6°C in Ringer's	8	663.63 ± 24.13	40.14 ± 3.69
24 hrs at 6°C in 2N Ca^{++} Ringer's	8	704.80 ± 42.48	39.79 ± 1.99
4 days at 6°C in normal Ringer's	8	636.22 ± 32.74	18.55 ± 1.51
4 days at 6°C in 2N Ca^{++} Ringer's	8	652.88 ± 30.47	19.32 ± 1.36
24 hrs at 6°C in zero Ca^{++} Ringer's	8	631.40 ± 45.91	21.21 ± 1.15
4 days at 6°C in Ringer's plus 10^{-5} g/ml NE	10	664.32 ± 32.71	23.12 ± 2.89
4 days at 6°C in Ringer's	10	682.13 ± 25.26	23.72 ± 2.66
24 hrs at 37°C in Ringer's	12	696.37 ± 55.33	40.12 ± 1.43

N   Number of aortas

a   aorta taken directly from animal for digestion

b   aorta dipped in normal Ringer's solution for few minutes (1-2) before digestion

c   mean value with standard error of the mean obtained after treatment shown

TABLE 4. Rabbit aorta calcium content after various treatments.

Condition	N	Calcium Content mEq/kg dry tissue
Fresh	12	14.03 ± 1.35 [a]
Vessels refrigerated 24 hours in normal Ringer's solution	15	47.46 ± 3.65
Vessels refrigerated 24 hours in zero Ca++ Ringer's solution	10	0 [b]
Vessels incubated 24 hours at 37°C oxygenated Ringer's solution	8	14.52 ± 1.77
Vessels refrigerated 24 hours in 2x Ca++ Ringer's solution	8	130.53 ± 10.20

Calcium^{-45} content of rabbit aortic tissue after various treatments

| Condition	N	Calcium^{-45} (moles Ca++/kg wet tissue/min)	Total Calcium Content mEq/kg wet tissue
Control 30' incubation with calcium-45 at 37°C	4	9.60 ± 0.71 [a]	4.33 ± 0.4 [a]
Refrigeration 24 hours in normal Ringer's solution and rewarmed to 37°C	4	0.32 ± 0.15	4.33 ± 0.5
Refrigeration 24 hours in 2x Ca++ Ringer's solution and rewarmed to 37°C	4	11.50 ± 0.20	4.19 ± 0.4

a   Mean value with standard error of the mean
N   Number of experiments
b   No calcium detected with our method

LEGENDS

Figure 1. Diagrammatic representation of apparatus used for perfusion of isolated branches of the dog femoral artery.

Figure 2. Effects of cold storage (24 hours, $6^{\circ}C$) on changes in flow through isolated dog blood vessels with changes in temperature. Ordinate: percent change in flow, control flow taken as 100%. Abscissa: temperature in degrees centigrade.

Figure 3. Effects of cold storage (24 hours, $6^{\circ}C$) and acute cold treatment (27° for $<$ 1 min.) on flow through isolated perfused dog blood vessels at temperatures from 37° to $45^{\circ}C$. Control flow is that obtained after cold storage and equilibration at $37^{\circ}C$ prior to the vessels being subjected to $27^{\circ}C$. Ordinate: percent change in flow, control flow taken as 100%. Abscissa: temperature in degrees centigrade.

Figure 4. Responses to norepinephrine of isolated perfused dog blood vessels at $37^{\circ}C$ (—) and $27^{\circ}C$ (---). N = vessels taken directly from dog and tested with norepinephrine. R = vessels refrigerated 24 hours at $6^{\circ}C$ and then tested. Ordinate: Resistance expressed as the reciprocal of flow. Pressure was held constant. Abscissa: Concentration of norepinephrine added to the bath.

Figure 5. Responses to norepinephrine of isolated perfused blood vessels at $37^{\circ}C$ (—) and $45^{\circ}C$ (---). N = vessels taken directly from dog and tested with norepinephrine. R = vessels refrigerated 24 hours at $6^{\circ}C$ and then tested. Ordinate: Resistance expressed as the reciprocal of flow. Pressure was held constant. Abscissa: Concentration of norepinephrine added to the bath.
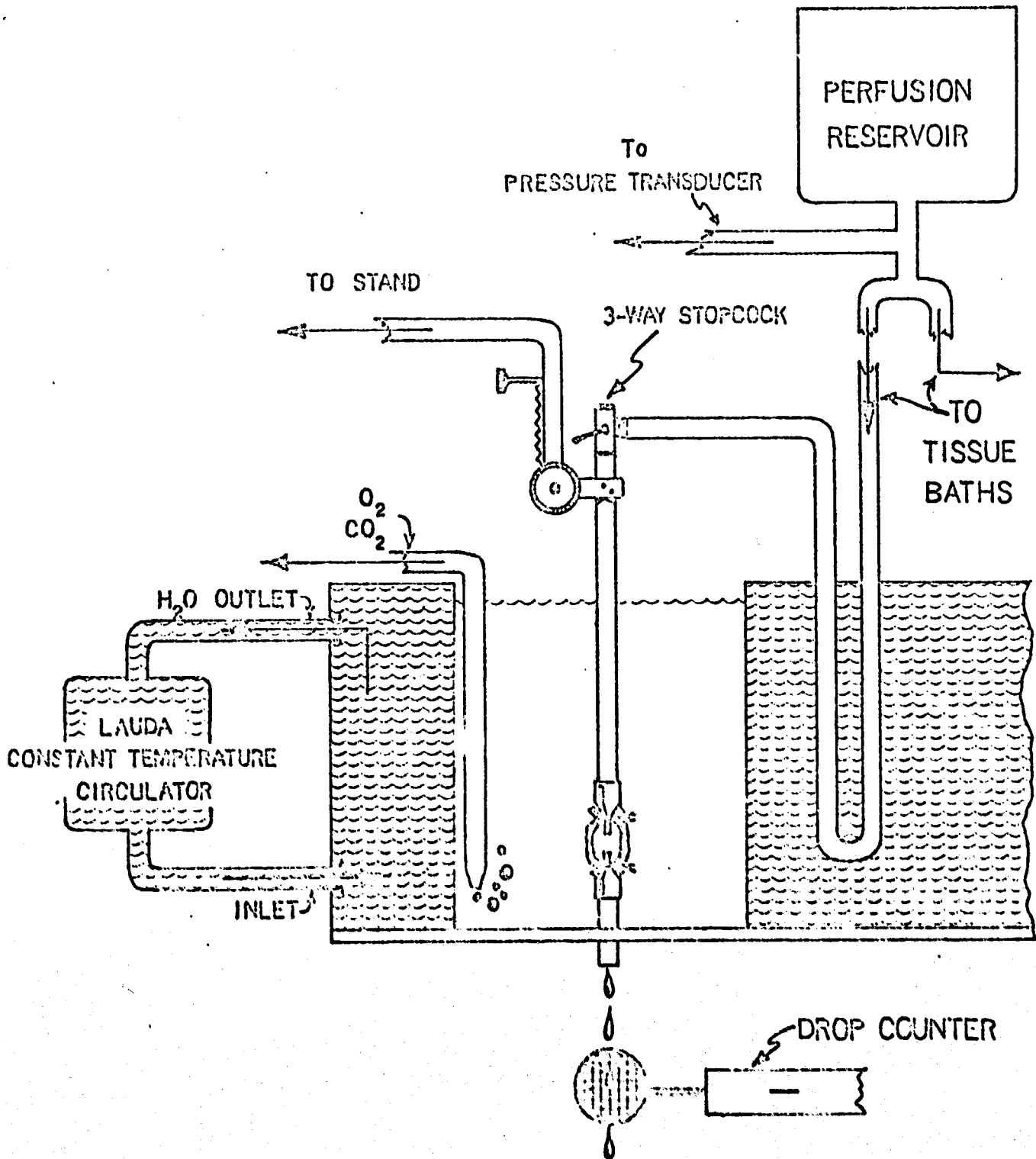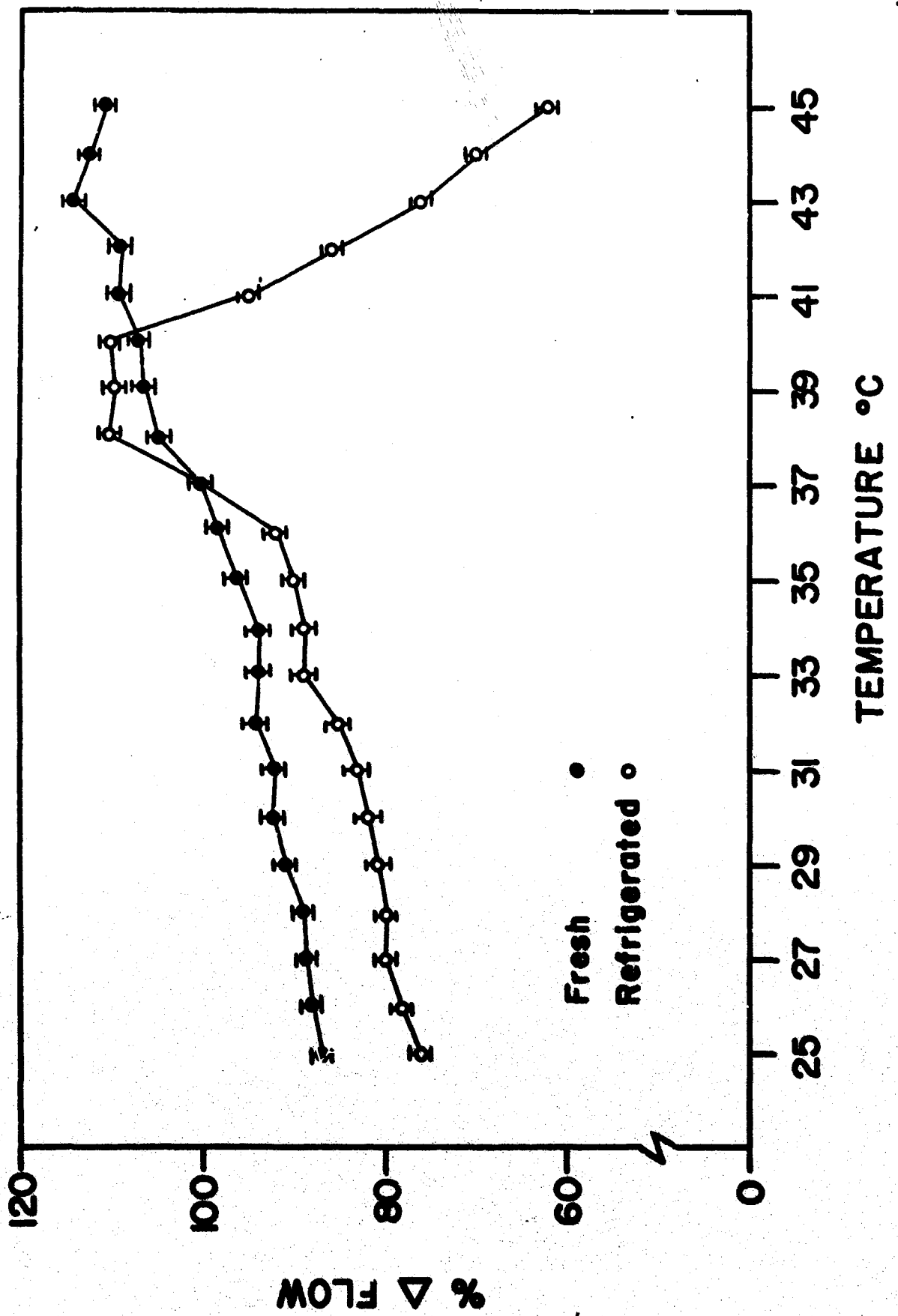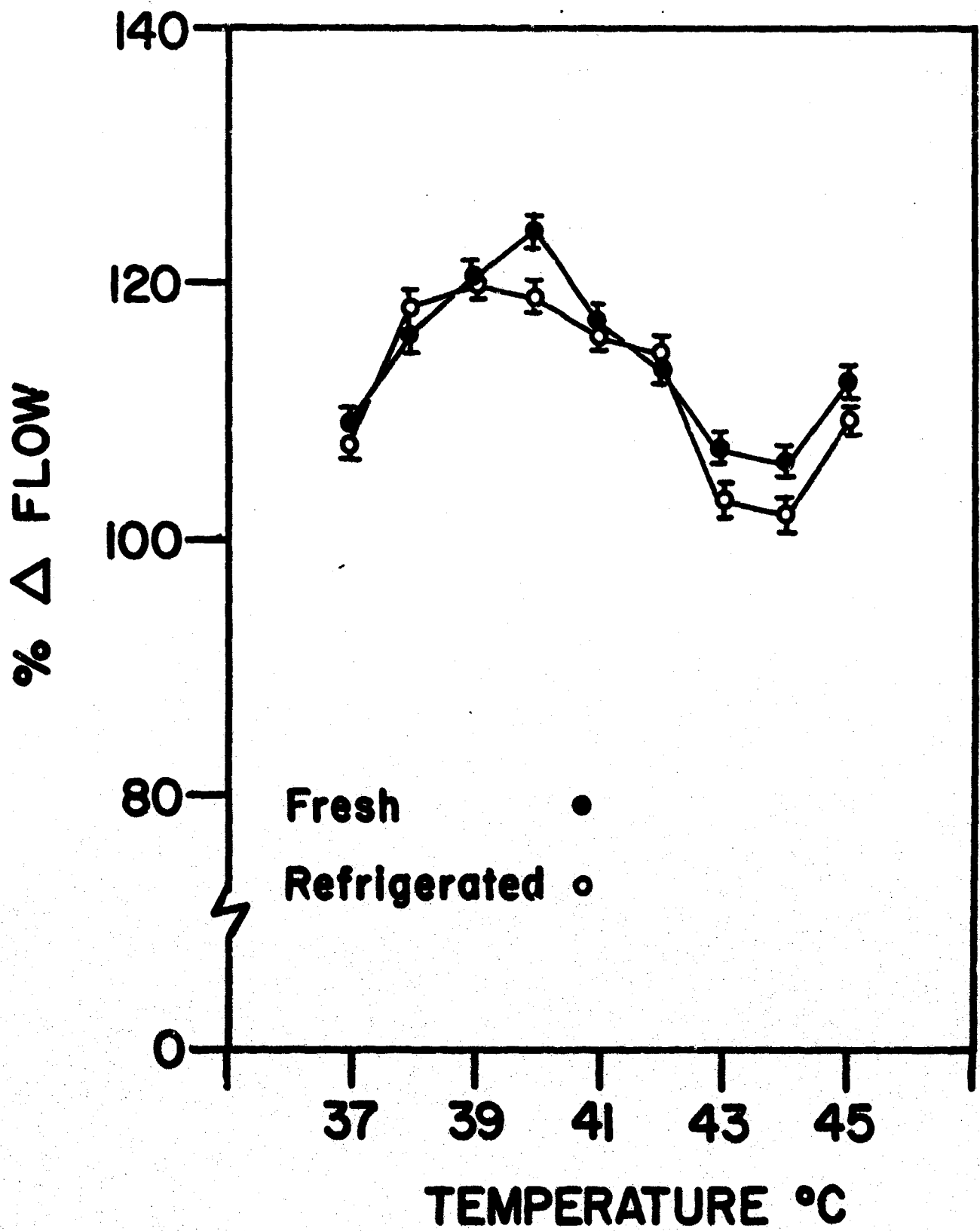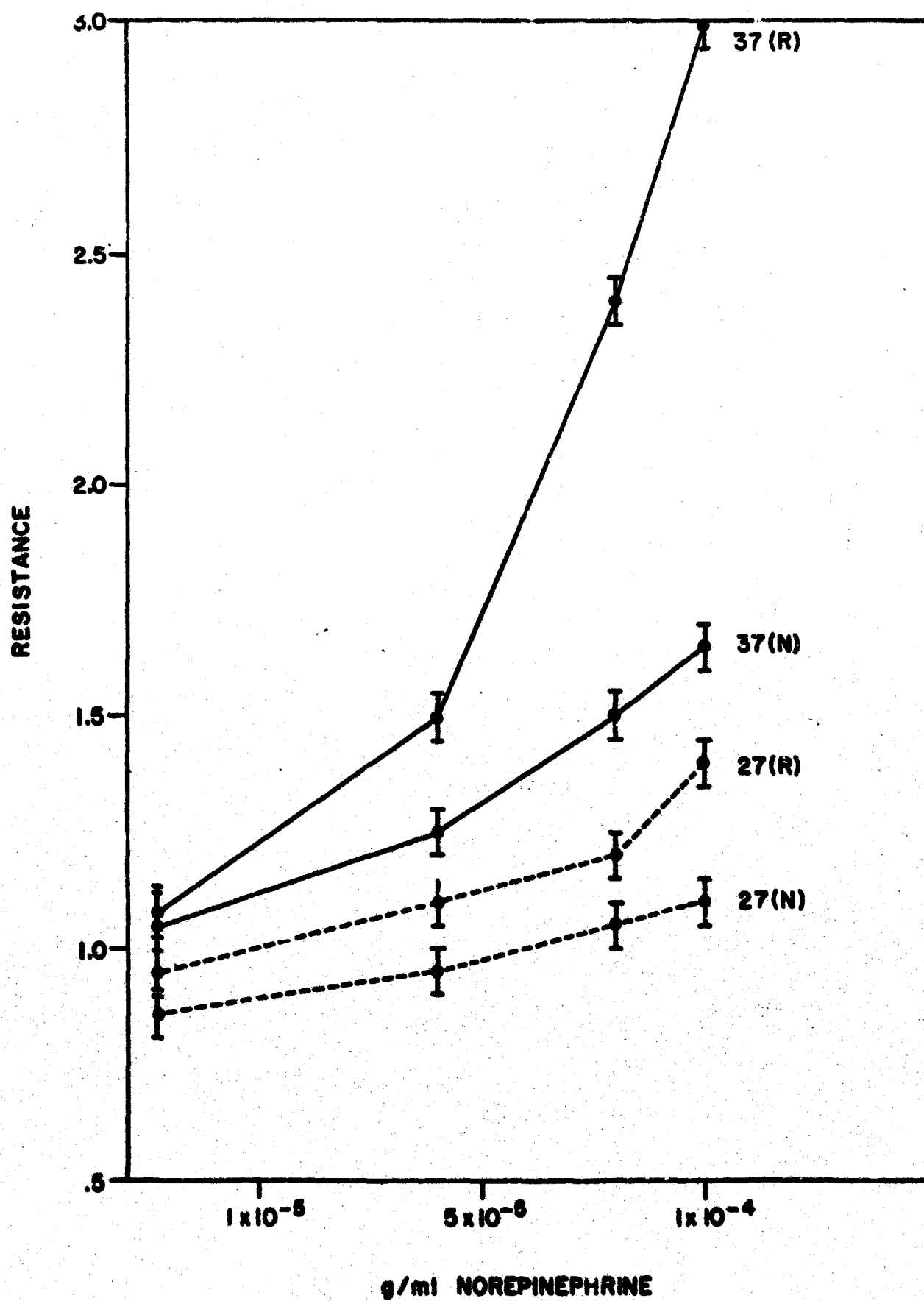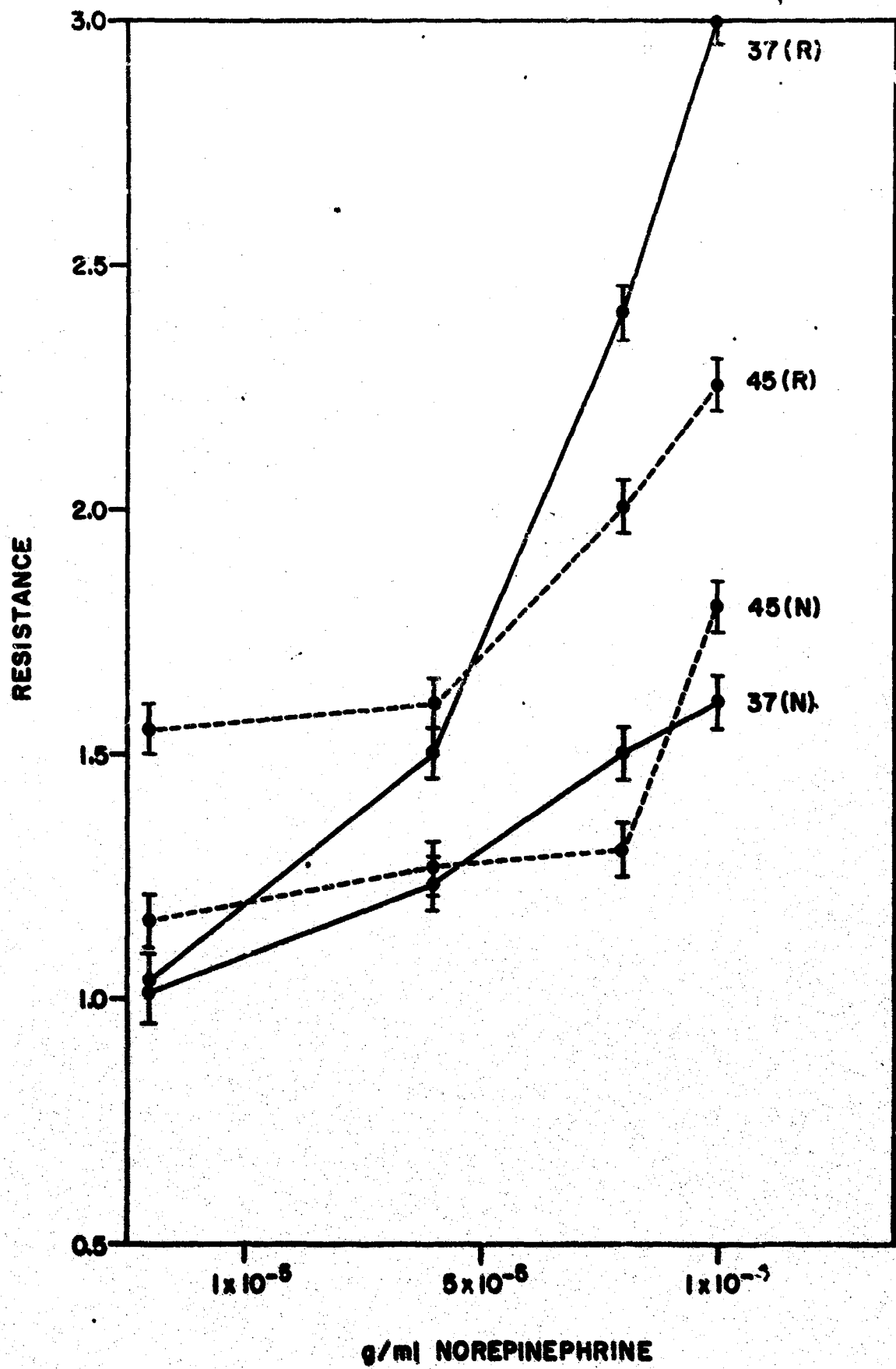
INTERACTION OF RESERPINE AND CALCIUM ON THE INOTROPIC

AND CHRONOTROPIC RESPONSES OF RABBIT ATRIA[1]

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ABSTRACT

Jurevics, Helga and Oliver Carrier, Jr. Interaction of reserpine and calcium on the inotropic and chronotropic responses of rabbit atria. J. Pharmacol. Exp. Ther. Isolated atria from young rabbits pretreated with 1 mg/kg and 3 mg/kg reserpine for 4 hours exhibited significantly greater tension responses to cumulative concentrations of calcium than atria from untreated rabbits. Propranolol was ineffective in reversing the enhanced inotropic responses to calcium. However, pretreatment with 3 mg/kg reserpine for 24 hours resulted in the contractile responses to calcium approaching those of control atria. Following equilibration in calcium-free Ringer's solution the atria pretreated with 3 mg/kg reserpine for 4 hours demonstrated (1) a decrease in the threshold concentration of calcium required to elicit a response, (2) an increase in the rate of tension change to calcium, and (3) a greater incidence of calcium-induced arrhythmias. The rate of tension decline by atria placed in calcium-free Ringer's solution was found to be significantly delayed following reserpine pretreatment ($t\frac{1}{2}$ = 63.14 seconds for control atria and 185.20 seconds for reserpine pretreated atria). No significant difference was found in the chronotropic responses to calcium of the reserpine pretreated and control atria. These findings demonstrate that calcium supersensitivity of the contractile response develops in rabbit atria following reserpine pretreatment for 4 hours and may result from both an increase in the membrane permeability to calcium and an alteration in the intracellular distribution of calcium.

Facilitation of the contractile response to various stimuli following reserpine pretreatment of the animal has been demonstrated in a number of _in vitro_ smooth muscle preparations. For example, reserpine pretreatment results in the development of a nonspecific supersensitivity to norepinephrine, acetylcholine, and potassium in rabbit aortic strips (Hudgins and Fleming, 1966); to norepinephrine, histamine, methylfurmethide, and potassium in the guinea-pig vas deferens (Westfall, 1970); and in perfused isolated small arteries from dogs to norepinephrine (Carrier and Holland, 1965) and calcium (Pegram and Carrier, 1969).

A nonspecific reserpine induced supersensitivity has also been demonstrated in cardiac muscle; however, these latter investigations have been primarily confined to the study of the chronotropic effect of various drugs (Trendelenburg and Gravenstein, 1958; Westfall and Fleming, 1968a and 1968b).

Reserpine induced supersensitivity to various stimuli has been extensively studied and is now believed to result either from a change in the physiological state of the responding cell beyond the receptor (Hudgins and Fleming, 1966; Westfall and Fleming, 1968) or to an alteration in the electrolyte distribution within the cell whereby more calcium is made available for contraction (Carrier and Shibata, 1967; Pegram and Carrier, 1969; Carrier, 1969).

A study by Nayler (1963) of the direct action of reserpine on isolated toad ventricular muscle revealed a depressant effect of reserpine on contractility which could readily be reversed by calcium,

caffeine and strophanthin-C. It was suggested that reserpine may exert an effect on cellular distribution of calcium. More recently, Hudgins and Harris (1970) demonstrated an increased efflux of calcium from supersensitive rabbit aortic strips following reserpine pretreatment. However, the total loss of calcium from the reserpine aortas was less than that lost from normal aortas.

A study of the action of reserpine on vascular tissue electrolytes (Carrier and Shibata, 1967; Carrier et al., 1967; Pegram and Carrier, 1969) revealed that this alkaloid is effective in causing an alteration in their sodium, potassium, and calcium contents. Since these cations play a role in muscle contraction, an alteration in their cellular distribution could result in an alteration in the response of the tissue when subjected to various stimuli. More recently, reserpine was also shown to effect an alteration in cardiac tissue electrolytes in the rabbit (Carrier et al., 1970). Burn and Rand (1958) found that atria from reserpine pretreated rabbits exhibited a significantly greater tension response following equilibration in McEwen's solution. However, no mention was made as to what could have produced this increased contractile response of the reserpine atria. The present study was undertaken to determine whether reserpine could effect a supersensitivity of the contractile response of the heart to calcium following pretreatment of young rabbits.

METHODS.

In vitro isolation. Albino rabbits of either sex, approximately 6 to 8 weeks of age were used in this study. Each animal was sacrificed

by a blow to the head and the whole heart was excised and placed in
oxygenated Ringer's solution (composition; NaCl, 154 mM; KCl, 5.4 mM;
$CaCl_2$, 2.4 mM; $NaHCO_3$, 6 mM; dextrose, 11 mM; in double distilled
deionized water; pH 7.4). Calcium-free Ringer's solution was pre-
pared as above with the addition of 10^{-5} M EDTA (Disodium(ethylenedinitrilo)
tetra acetate) but contained no $CaCl_2$. The spontaneously-beating right
and left paired atria were isolated and placed in a tissue-organ bath
with a final volume of 75 ml. The Ringer's solution in the bath was
continuously oxygenated with 95% oxygen -5% carbon dioxide and maintained
at a constant temperature of $31^{o}C$. Immediately upon placing the atria
in the bath, a tension of 1 gram was applied. The atria were allowed
to equilibrate for approximately 1 hour or until a constant contractile
tension and rate were maintained. Isometric contractions and heart
rate responses were measured with a Grass FT-03 force-displacement
transducer and recorded on a Grass Model 7 polygraph.

Responses to calcium. Contractile responses to various concen-
trations of calcium were obtained by a cumulative increase in the
total concentration of calcium in the bath. Before each successive
concentration of calcium was added, the atria were allowed to reach a
new steady tension or were allowed to respond for 15 minutes, particularly
in the case of atria subjected to less than 2.4 mM calcium. In the ex-
periments using propranolol (Inderal, Ayerst Laboratories) the atria,
after an initial 1 hour equilibration in normal-calcium Ringer's solu-
tion, were equilibrated an additional 45 minutes in the presence of pro-
pranolol before contractile responses to calcium were determined.

Initial experiments had established that 10^{-5}M propranolol resulted in an approximately 3 log unit shift to the right of the normal isoproterenol dose-response curve on both contractile tension and heart rate. The sensitivity of the pacemaker to calcium was determined by the concentration of calcium producing a persistent arrhythmia; that is, an arrhythmia which did not reverse spontaneously within 10 seconds.

Rates of tension responses to calcium. Control and reserpine pretreated atria (3 mg/kg for 4 hours) were placed in Calcium-free Ringer's solution containing 10^{-5}M EDTA. Over a period of 30 to 40 minutes the control atria declined to a new contractile tension of approximately 0.03g and the reserpine pretreated atria declined to 0.05g. The rate constants (k), for the decline in tension with time were determined according to the method of Holland (1966). He found that stimulated rabbit atria when transferred to a low calcium medium exhibited an exponential decline in contractile tension with time which could be expressed by the equation $T = e^{-(kt)}$, where T is the normalized contractile tension obtained by subtracting the new equilibrium tension from the initial tension at time = 0; k is the specific rate constant in seconds^{-1}; and t is the time in seconds. The fast component in the decline in contractile tension was obtained by plotting the difference between the initial decline in tension and the slow component of the decline in tension. The specific rate constant, k, of the fast component was obtained from the equation $k = .693/t\frac{1}{2}$. Since the rate of rise in contractile tension following

drug exposure cannot be expressed as a simple exponential function with time (Holland, 1966), the time to one-half maximum contractile tension with each cumulative concentration of calcium was used as an index for the rate of contractile response to calcium by control and reserpine pretreated atria.

Pretreatment schedule and statistical evaluation. All injections of reserpine (Serpasil, Ciba Pharmaceutical Products, Inc.) were administered in a single dose of either 1 mg/kg or 3 mg/kg intraperitoneally either 4 or 24 hours before the beginning of each experiment. All statistical evaluations were performed by Student's t-test or chi-square analysis.

RESULTS.

The first phase of this study was the comparison of the contractile response of normal and reserpine pretreated atria to cumulative concentrations of calcium. Rabbits were pretreated with 1 mg/kg and 3 mg/kg reserpine for 4 and 24 hours. All contractile respo s are expressed in grams tension. Figure 1 shows that both 1 mg/kg and 3 mg/kg reserpine pretreatment for 4 hours resulted in the reserpine atria developing a significantly greater contractile response to cumulative concentrations of calcium up to approximately 9.6 mM calcium. Further increase in the concentrations of calcium resulted in no significant difference in the tension responses of the atria pretreated with 1 mg/kg reserpine and the control atria. However, the contractile responses of atria pretreated with 3 mg/kg reserpine for 4 hours were significantly greater than the control atria up to 12 mM calcium (Fig. 1). The inotropic

responses of both groups of reserpine pretreated atria were not
significantly different from each other. As shown by the curves
in Figure 2, the contractile responses to calcium of atria pretreated
with 3 mg/kg for 24 hours were not significantly different from the
responses of control atria.

Since the atria pretreated with reserpine for 4 hours
exhibited a greater contractile tension than control atria follow-
ing equilibration in Normal-calcium Ringer's solution, we were
interested in seeing if this same effect could be demonstrated in
Ringer's solution containing less than 2.4 mM calcium. Figure 3
shows the contractile responses of control and reserpine pretreated
(3 mg/kg for 4 hours) atria to cumulative calcium concentrations
following an initial equilibration in Calcium-free Ringer's solution.
The contractile responses of the reserpine pretreated atria were sig-
nificantly greater than the responses of control atria to all concen-
trations of calcium. The reserpine atria also demonstrated a decrease
in the threshold concentration of calcium needed to elicit a response,
and exhibited a greater maximum contractile tension. The effect of
calcium on the heart rate of control atria and atria pretreated
with 3 mg/kg reserpine for 4 hours is illustrated in Table 1. Although
the reserpine pretreated atria exhibited a slightly lower heart rate
in the presence of calcium than the control atria, the heart rate re-
sponses of the control and reserpine pretreated atria were not sig-
nificantly different over the concentration range of calcium employed
in this study. The inability to demonstrate supersensitivity to calcium
of the chronotropic response in the in vitro preparations has also

been reported by Westfall and Fleming (1968).

Since reserpine pretreatment with 3 mg/kg for 4 hours does not result in complete depletion of catecholamines from the rabbit heart, and since calcium has been suggested to be a mediator in the release of catecholamines from presynaptic storage sites (Hukovic, 1962), there is the possibility that the supersensitivity to calcium observed in the reserpine atria could be mediated through calcium's release of norepinephrine, and the supersensitivity seen with calcium could, in fact, be norepinephrine supersensitivity. To determine if this was possible, the contractile responses to increasing concentrations of calcium of control and reserpine pretreated atria were determined in the presence of 10^{-5} M propranolol. In Figure 4 it can be seen that propranolol was ineffective in reversing the enhanced contractile response of the reserpine pretreated atria to cumulative concentrations of calcium. However, at this concentration, propranolol did exhibit a slight depressant effect on the myocardium. The depression of the contractile tension in Normal-calcium Ringer's solution was $18.16 \pm .36\%$ for control atria and $14.35 \pm .60\%$ for the reserpine pretreated atria. However, the inability of propranolol to reverse the potentiation of the contractile response to calcium in the reserpine pretreated atria, along with the relatively constant heart rate responses with cumulative calcium concentrations, would indicate that calcium was exerting an action independent of a mechanism involving the release of endogenous catecholamines.

The number of control atria and atria pretreated with 3 mg/kg reserpine for 4 hours which developed arrhythmias to cumulative

calcium concentrations are given in Table 2. The reserpine pretreated atria were found to be significantly more sensitive than control atria to calcium induced arrhythrias as determined by chi-square analysis.

The rate of decline in contractile tension in Calcium-free Ringer's solution by control atria and reserpine atria following 3 mg/kg pretreatment for 4 hours is illustrated in Figure 5 and Table 3. Although the reserpine pretreated atria exhibited an initially greater contractile tension, $1.80 \pm .09$ g, than control atria, $1.44 \pm .11$ g $(P<.05)$, the rate of the fast component in tension decline, k, of the reserpine pretreated atria in Calcium-free Ringer's solution was significantly decreased $(P<.01)$. No significant difference in the rate of decline of the slow component, in tension, k', was found between control and reserpine pretreated atria $(P<.2)$. The decrease in the rate of tension decline of reserpine pretreated atria placed in Calcium-free Ringer's solution suggests that some intracellular fraction of calcium involved with contraction was being either retained by the atria or was being utilized more efficiently.

The effect of reserpine pretreatment on the time to half maximum tension with cumulative concentrations of calcium is illustrated in Table 4. The times to half maximum tension of both control atria and atria pretreated with 3 mg/kg reserpine for 4 hours in response to 0.6 mM and 1.2 mM calcium were not significantly different; however, with 1.8 mM calcium and increasing concentrations the reserpine atria exhibited a significantly greater rate of response to calcium.

DISCUSSION.

The results obtained in the present study demonstrate that super-

sensitivity of the contractile response to calcium develops in rabbit atria following pretreatment with 3 mg/kg reserpine for 4 hours. However, 24 hour pretreatment with 3 mg/kg reserpine results in the reserpine atria approaching the response of control atria to cumulative concentrations of calcium. The inability of propranolol to block the enhanced contractile responses of the reserpine pretreated atria to calcium along with the relatively constant chronotropic responses to calcium indicates that the calcium supersensitivity of the contractile response is not mediated through a mechanism involving the release of catecholamines. These findings and the findings of Carrier et al. (1970), that reserpine causes a reduction in calcium content of rabbit hearts within 4 hours, indicate that reserpine may be exerting an action through a mechanism involving an alteration in the distribution of intracellular or membrane calcium.

The ability of calcium to alter cell membrane stability is well known (Brink, 1954; Shanes, 1958) and the possibility that reserpine pretreatment may result in an increase in cell membrane permeability through its effect on calcium has been suggested previously (Carrier and Shibata, 1967). That this may be true is indicated in the present study by the increase in the sensitivity of the reserpine atria to calcium-induced arrhythmias. Holland and Tinsley (1958) reported that some drug-induced arrhythmias can result from an increase in cell membrane permeability. Also, Westfall and Fleming (1968b) found that reserpine pretreatment of guinea-pigs resulted in an increase in the sensitivity of the in vivo heart to norepinephrine-induced arrhythmias.

The ability of microsomal and mitochondrial lipid fractions to

promote the transport of ionized calcium across an aqueous-lipid
solvent interphase indicates that lipids present in the membrane
are involved with calcium ion movements (Nayler, 1966). The beta
adrenergic blocking drugs, propranolol and pronethalol (Nayler, 1966),
as well as reserpine (Baltzer, 1968b), have been shown to bind to
membrane lipid fractions and to alter the rate of calcium transport.
It is possible that the depression of cardiac contractility (Ziamus,
1961) or the increase in responsiveness to drug stimulation observed
following reserpine pretreatment of the animal results from reserpine
interacting with a membrane lipid component whereby a structural
change in the membrane is induced and the calcium equilibrium is
altered.

A redistribution of intracellular calcium induced by reserpine
is further evidenced by the decrease in the threshold requirement
of calcium to induce a positive inotropic response by atria following
pretreatment with 3 mg/kg reserpine for 4 hours. In view of this
one might suspect that the initial tissue calcium depleting action
observed with reserpine (Carrier and Shibata, 1967) could be due to
an alteration in the balance between bound and free calcium resulting
in a redistribution between cellular and interstitial calcium.

The hypothesis that reserpine may have an effect on calcium
distribution inside the cell is in general agreement with several
recent findings. Baltzer, et al., (1968a) found that both calcium
induced increase in ATPase activity and the rate of uptake of calcium
by vesicular fragments of the sarcoplasmic reticulum from rabbit skeletal
muscle were reduced by reserpine; however, the calcium storing capacity

and the ability to concentrate calcium by these vesicular fractions were not inhibited by reserpine. Hudgins and Harris (1970) found that while in reserpine-induced supersensitive aortas, calcium efflux is increased initially, the total content of calcium remaining in the reserpine tissues was greater than that in non-treated aortas following soaking in Calcium-free Ringer's solution. If data obtained on aortas could be extrapolated to hearts, then the decrease in the rate of tension decline by the reserpine pretreated atria observed in this study would suggest that these atria were in essence retaining calcium by losing it at a slower rate to the Calcium-free Ringer's solution, or that intracellular calcium was being redistributed in such a manner as to make it more efficient for utilization in contraction. Also in the present study, the reserpine atria were observed to exhibit an increase in the rate of response to calcium. This would indicate that the reserpine atria either exhibited an increased influx of calcium due to an alteration in membrane permeability, or that some intracellular pool of calcium which is intimately associated with the contractile proteins is made more readily available for contraction. This could be accomplished by a modification in the rate of uptake of calcium by the sarcoplasmic reticulum.

CONCLUSION

Supersensitivity of the contractile response to calcium develops in isolated rabbit atria following pretreatment with 1 mg/kg and 3 mg/kg reserpine for 4 hours, but not after 24 hours pretreatment. Although the heart rate responses to calcium were not affected by reserpine pretreatment for 4 hours, the atria did show an increased sensitivity to calcium-induced arrhythmias. This latter effect indicates that an increase in membrane permeability has developed following reserpine pretreatment. The ability of reserpine to induce an alteration in calcium metabolism in cardiac tissue is reflected by changes in certain physiological parameters. These are an increased incidence of arrhythmias, an increased rate of tension response to calcium, and a delay in the rate of tension decline of atria subjected to a calcium deficient medium. Therefore, on the basis of these findings, it is possible to suggest that reserpine-induced supersensitivity to calcium in rabbit atria results from an alteration in membrane permeability and a redistribution of intracellular calcium whereby more calcium is made available for contraction.

ACKNOWLEDGEMENTS

FOOTNOTE

TABLE 1

The effects of reserpine pretreatment (3 mg/kg for 4 hours) on the heart rate responses to calcium of isolated rabbit atria

Total Calcium in Medium	0.60 mM	1.20 mM	1.80 mM	2.4 mM	3.60 mM	4.80 mM	7.20 mM	9.60 mM	12.00 mM
Control Atria (10)[a]	95.4±3.6[b]	97.2±3.9	95.1±3.9	92.9±2.9	90.5±2.8	88.2±3.3	87.5±3.2	85.5±3.8	85.8±4.1
Reserpine Atria[c] (10)	87.6±2.7	36.1±3.3	87.3±4.0	86.9±4.7	84.4±4.8	84.5±6.6	82.9±6.0	83.2±6.0	79.2±2.9

a   Number in parenthesis represents number of atria

b   Mean heart rate ± SEM in beats/minute

c   Not significantly different from control

TABLE 2

Concentration of calcium resulting in arrhythmias in untreated rabbit atria and in rabbit atria pretreated with 3 mg/kg reserpine for 4 hours

	NUMBER OF ATRIA DEVELOPING ARRHYTHMIAS FOLLOWING A CUMULATIVE CALCIUM CONCENTRATION OF:									
	0.6mM	1.2mM	1.8mM	2.4mM	3.6mM	4.8mM	7.2mM	9.6mM	12.0mM	14.4mM
CONTROL ATRIA						1	1	1	3	3
RESERPINE ATRIA*	2	1			2	1		2	2	

* Significantly different from control by chi-square analysis (P $<$ .01)

TABLE 3

Effect of reserpine pretreatment (3 mg/kg for 4 hours) on the specific rate constant k and t½ of the fast component of tension decline, and on k' and t½' of the slow component of tension decline of rabbit atria in Calcium-free Ringer's solution[a]

	N	$t\frac{1}{2} \pm SEM$ sec	$k \pm SEM \times 10^{-2} sec^{-1}$	$t\frac{1}{2}' \pm SEM$ sec	$k' \pm SEM \times 10^{-2} sec^{-1}$
CONTROL ATRIA	7	63.1±7.0	1.196±.161	718.6±130.8	.114±.107
RESERPINE ATRIA	6	185.4±38.1	.447±.077	1063.3±171.1	.076±.014
P - VALUE		<.05	<.05	<.2	<.2

[a] Values for t½, k, t½', and k' were obtained from the relationship $T = e^{-(kt)}$

TABLE 4

Rate of response of normal and reserpine (3 mg/kg for 4 hours) pretreated atria to half maximum tension to cumulative concentrations of calcium

	N	Total cumulative concentration of calcium							
		0.6mM	1.2mM	1.8mM	2.4mM	3.6mM	4.8mM	7.2mM	9.6mM
CONTROL ATRIA	12	302.0±13.5*	281.7±17.4	268.5±24.8	220.0±20.0	134.3±16.1	107.9±10.3	37.7±15.1	67.5±6.07
RESERPINE ATRIA	12	254.0±44.0	258.6±26.8	191.6±26.8	137.2±16.1	80.0±10.0	64.1± 8.3	48.6±16.0	45.3±15.8
P – VALUE		<.05	<.60	<.05	<.01	<.02	<.01	<.05	<.02

* Mean response ± SEM in seconds

LEGENDS

FIG. 1: Mean contractile responses to cumulative concentrations of calcium of untreated rabbit atria and rabbit atria following pretreatment with 1 and 3 mg/kg reserpine for 4 hours. One hour equilibration in 2.4mM calcium Ringer's solution preceeded the calcium determination. Vertical bars represent standard error of the mean. N = number of experiments.

FIG. 2: Mean contractile response to cumulative concentrations of calcium of untreated rabbit atria and rabbit atria following pretreatment with 3 mg/kg reserpine for 24 hours. One hour equilibration in 2.4mM calcium Ringer's solution preceeded the calcium determination. Vertical bars represent standard error of the mean. N = number of experiments.

FIG. 3: Mean contractile responses to cumulative concentrations of calcium of untreated rabbit atria and of rabbit atria following pretreatment with 3 mg/kg reserpine for 4 hours. The atria were equilibrated in Calcium-free Ringer's solution containing 10^{-5}M EDTA before responses to calcium were determined. Vertical bars represent standard error of the mean. N = number of experiments.

FIG. 4: Effect of propranolol on the contractile response of untreated rabbit atria and rabbit atria pretreated with 3 mg/kg reserpine for 4 hours to cumulative concentrations of calcium. Following the initial 1 hour equilibration in 2.4mM calcium, the atria were equilibrated an additional 45 minutes in the presence of 10^{-5}M propranolol before responses to calcium were determined. Each point represents the mean response and vertical bars the standard error of the mean. N = number of atria.

FIG. 5: Semilogrithmic plot of the relative decline in contractile tension with time in Calcium-free Ringer's solution of untreated rabbit atria and rabbit atria following pretreatment with 3 mg/kg reserpine for 4 hours. Each point represents the mean of six to seven atria.
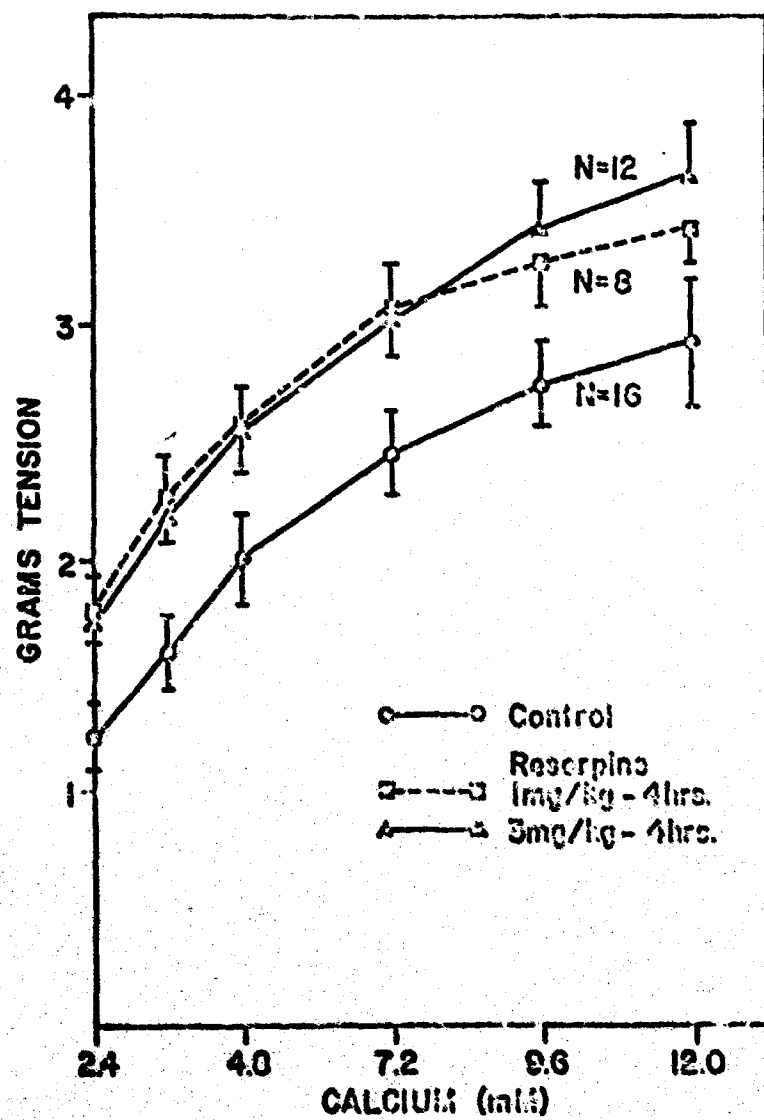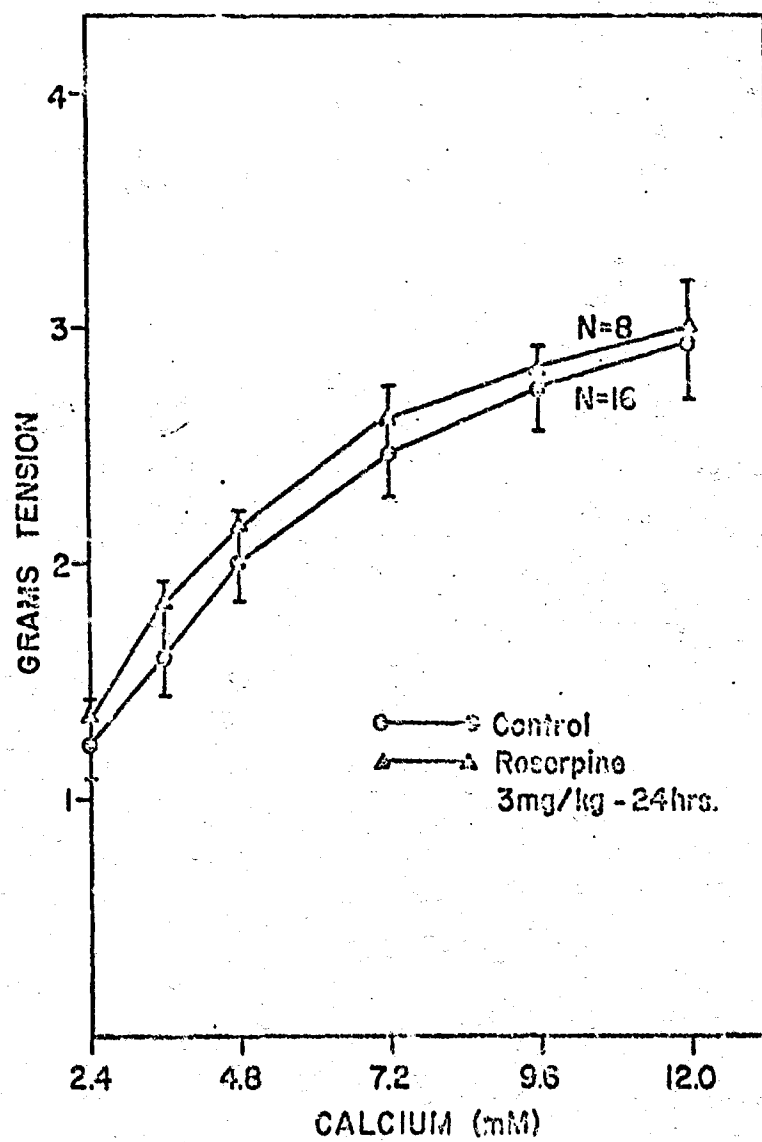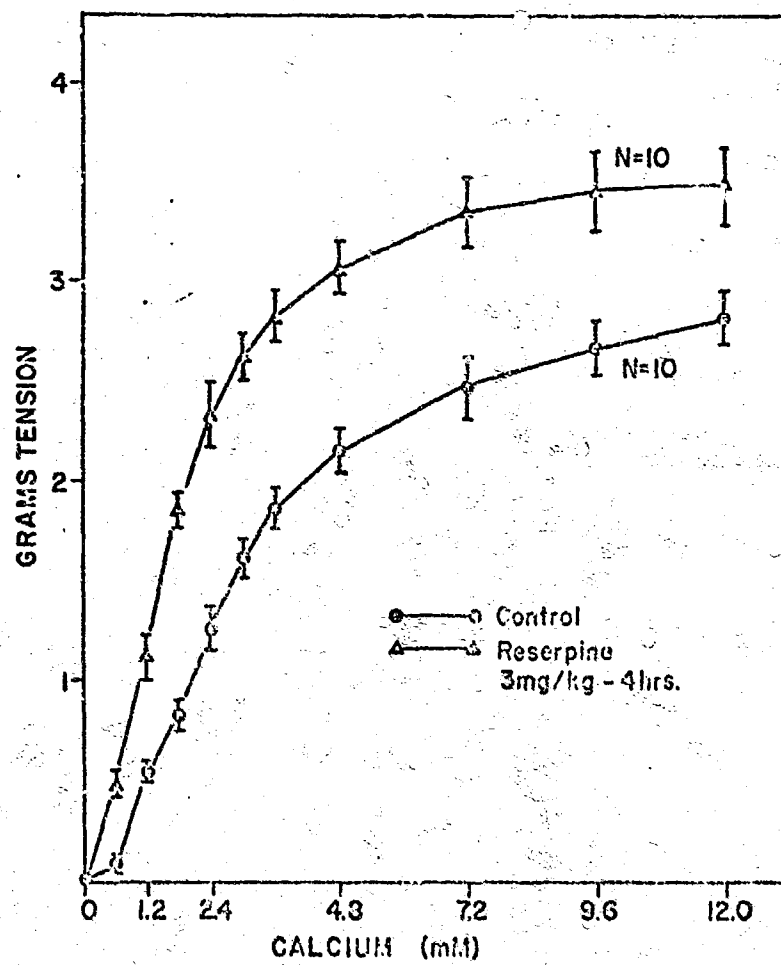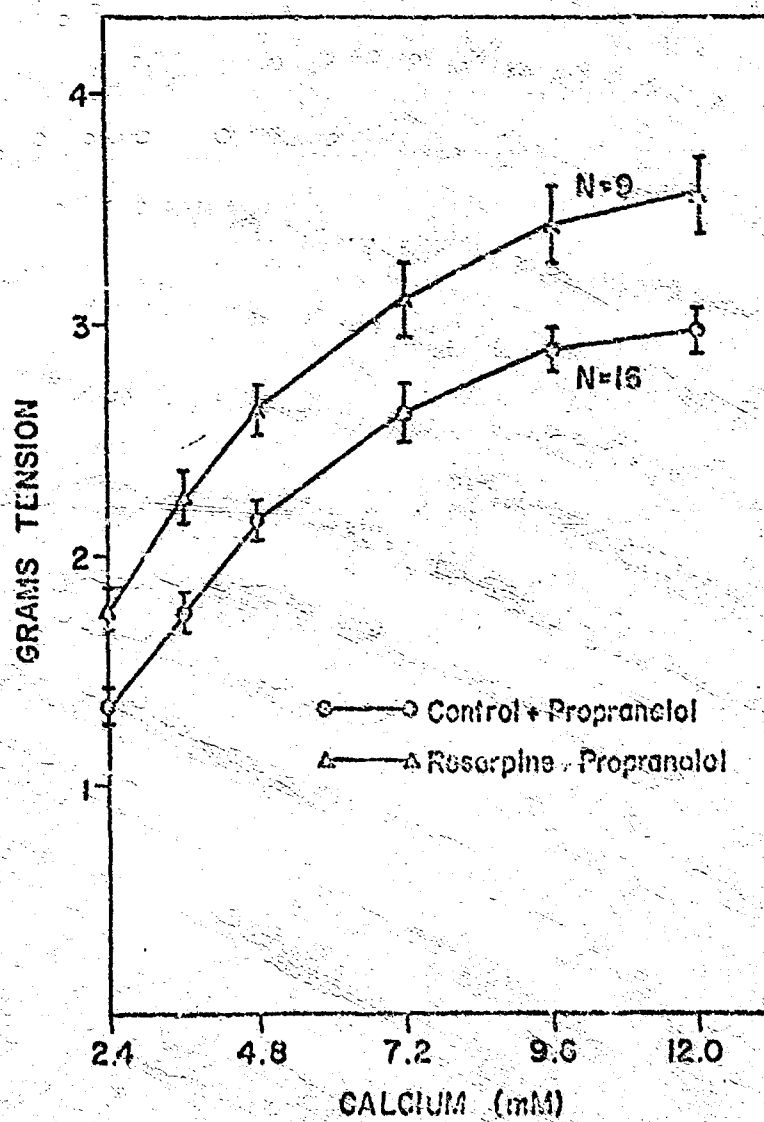
FIGURE 1

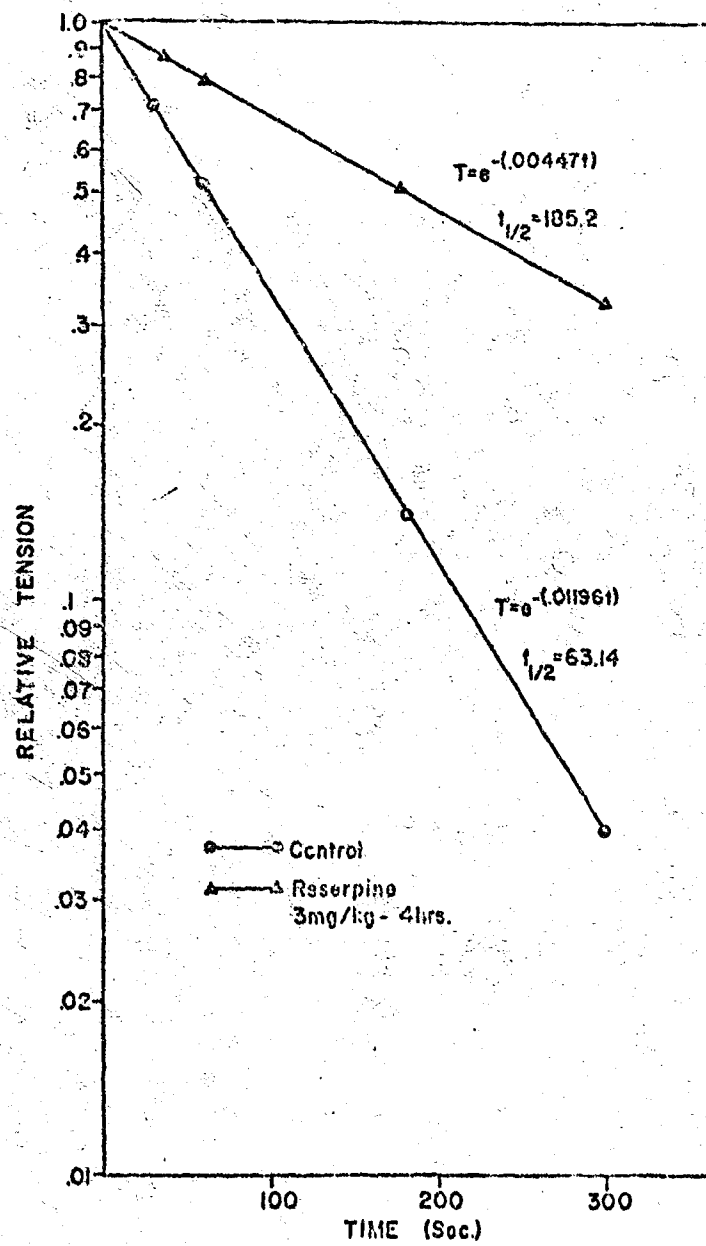FIGURE 2

FIGURE 3

FIGURE 4

FIGURE 5

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In the past year experiments were performed to ascertain whether PGE_1 and $PGF_{2\alpha}$ exerted effects on Ca transport in the heart and whether it was possible to correlate this at the pharmacological and biochemical levels. The two models studied were the rate controlled guinea pig left atria and cardiac sarcoplasmic reticulum (SR). These models were selected because of the strong evidence from a number of laboratories intimately linking Ca exchange by cardiac muscle and the strength of contraction.

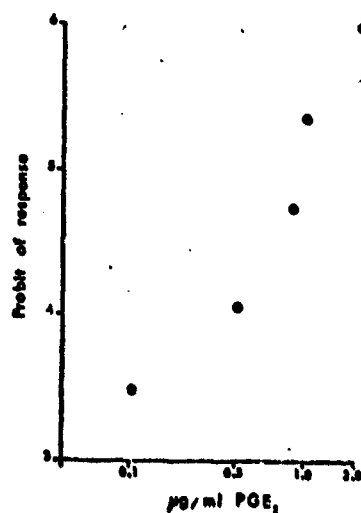Using the isolated guinea pig atria the effects of the prostaglandins on contractility, tissue Ca and Ca^{45} exchange were determined. Figure 1 is a probit plot demonstrating the effects of PGE_1 on maximum tension development following 10 minutes incubation. At 1 µgm/ml tension increases $62.3 \pm 8.0\%$ ($\pm$S.E.); the $PGF_{2\alpha}$ curve falls below this at all concentrations tested and the final results were highly variable (for example, 1 µgm/ml increased tension $35.9 \pm 19.5\%$). If this same experiment is done on the spontaneously beating heart PGE_1 increases tension 30.0%, and markedly increases heart rate and coronary flow. This effect on coronary flow is quite pronounced and persists for about 30 minutes.

FIGURE 1

Figure 2 summarizes the results obtained following a 10 minute incubation with the prostaglandins on total tissue Ca content and Ca^{45} exchange. It can

The effect of PGE_1 and PGF_{2a} (1 μg/ml) on tissue calcium content and Ca^{45} exchange in guinea pig atria stimulated at 2 cps.

	Tissue Calcium [a] μeq/kg tissue x 10^3 ±S.E.	Calcium[45] Uptake μeq/kg tissue/min x 10^4 ±S.E.	Relative Calcium[45] Efflux (10 min)
Ethanol	15.991 ± 1.550 (11)	1.042 ± 0.181 (4)	1.0 (4)
PGE_1	17.779 ± 2.010 (7)	4.456 ± 0.269** (4)	1.43* (4)
PGF_{2a}	17.629 ± 2.010 (7)	3.396 ± 0.417* (4)	1.36* (4)

* $p < 0.05$
** $p < 0.001$

[a] dry weight; at 76.762 H_2O the values are 3.824 (±0.369), 4.044 (±0.467), and 4.143 (±0.513) respectively.

FIGURE 2

be seen that total Ca did not significantly change from control as measured by atomic absorption. The trend toward increase did not show significance and with the sample weights we are operating at the lower end of the sensitivity curve for Ca in aqueous solutions. There was, however, a significant increase in Ca^{45} uptake at the time when the increase in contractility was maximum. If the tissue is loaded for 2 hours in Ca^{45} medium and then transferred to an unlabeled medium containing the prostaglandins enhance the rate of efflux from the tissue. Further analyses indicate that at least 2 separate Ca compartments are involved, and it is the rapidly exchangeable compartment ($t\frac{1}{2}$ = 3 min.) that is significantly altered by the prostaglandins. Prior experiments by Klaus and Piccinini (Experientia, 23:556, 1967) by indirect observations suggested that the Ca uptake was responsible for the pharmacologic effect, however, their data are subject to certain criticisms.

Separate experiments were carried out to determine whether the prostaglandins affected an intracellular source of Ca. In these experiments ventricles were excised and homogenized in isotonic sucrose containing 5mM sodium azide. The homogenate was passed through a sucrose gradient (0.3→1.0M) using a Beckman L2-65B ultracentrifuge at 0°C. Azide was added to inhibit any Ca transport by mitochondria, a contaminant in this preparation of about 2-3%. The appropriate experiments were performed indicating that azide does not affect the resulting SR, and that under our conditions purified preparations of mitochondria

will not accumulate significant amounts of Ca. The SR was incubated 10 minutes in an imidazole buffer, pH 7.0, containing 5mM ATP, 5mM Mg, 5mM K-Oxalate, 100mM KCl, and 0.1mM $Ca^{45}Cl_2$ (final protein, 0.1 mgm/ml). At the end of the incubation period an aliquot was passed through a Millipore filter (0.45 μ pore diameter), and the amount of Ca taken up by the SR was calculated.

The effect of PGE_1 and $PGF_{2\alpha}$ on Ca^{45} uptake by fragmented cardiac sarcoplasmic reticulum.

	N	Time (min)	μmol Ca/mgm protein ±S.E.	
Ethanol	4	10	0.850±0.003	
PGE_1 (1μgm/ml)	5	10	0.972±0.004	P<0.01
$PGF_{2\alpha}$ (1μgm/ml)	5	10	0.968±0.010	P<0.01

0.1 mgm protein/ml

FIGURE 3

Figure 3 summarizes these results; both PGE_1 and $PGF_{2\alpha}$ significantly increased Ca uptake by the SR. To test the possibility that this effect may be due to a nonspecific action of fatty acids, experiments were done using arachadonic and stearic acids. In both instances Ca uptake was depressed.

If efflux curves are plotted, that is, allow the SR to accumulate Ca for 10 minutes in the presence of the prostaglandins, filter an aliquot, and wash the filter 5 times during a 10 minute period with a solution containing the prostaglandins and 0.1mM EGTA, Ca efflux is enhanced significantly compared to control with the most marked effects occurring between 2 and 6 minutes. These data correlate in a temporal and quantitave manner quite nicely with the whole tissue (Sabatini-Smith, S., Pharmacol. 12:339, 1970).

No satisfactory theory has been proposed to explain the diverse effects of the prostaglandins. Due to the fact that they lower muscle cell membrane threshold to electrical stimulation, it is possible that they act at the cell membrane and within the cell itself. These data would indicate that both PGE_1 and $PGF_{2\alpha}$ augment myocardial contractility by increasing intracellular Ca

stores, thus enhancing Ca availability in the vicinity of the troponin-tropomyosin proteins. The question of whether this effect on Ca transport can be extended as a basic physiological mechanism of action for the prosta-glandins remains to be answered. Certain recent evidence from other tissues would suggest this is true (Ramwell, P. and J. Shaw, Eds., New York Acad. Sci. Symp., "Prostaglandins" Setp. 1970, in press).

(1) AN EVALUATION OF THE CHANGES IN LEFT VENTRICULAR DIAMETER WITH THE RESPECT
TO PRESSURE DURING DIASTOLE (Lawrence D. Horwitz and Vernon S. Bishop).

The purpose of this study was to describe the dynamic function of the left
ventricle in terms of its diameter, pressure and outflow at rest and during
infusions of isoproterenol and metaraminol in conscious, unsedated dogs. Special
attention was focused on the nature of the diastolic stress-strain pattern of
the ventricle and its influence on filling.

Isoproterenol induced tachycardia, a decrease in left ventricular end-diastolic
and end-systolic diameters, and a decrease in left ventricular end-diastolic
pressure; metaraminol induced bradycardia, increase in end-systolic and end-
diastolic diameters and an increase in end-diastolic pressure. Neither drug
altered stroke volume in a significant manner. Dp/dt and circumferential
shortening rate, measurements commonly cited as indicative of myocardial con-
tractile strength, increased with isoproterenol and were unaffected by metaraminol.
Another measurement which has been used by some investigators as an index of con-
tractility, dP/dt, did not change significantly with isoproterenol and decreased
with metaraminol.

From these studies it appeared that the heart counteracts an increase in
afterload by an increasing end-diastolic size, whereas beta-adrenergic stimu-
lation is characterized by production of the same stroke volume from an end-
diastolic volume less than that of the control by means of a more complete con-
traction and a smaller end-systolic volume.

The tachycardia effect of isoproterenol does not account for the changes
seen in diameter flow or dP/dt. Other studies in our laboratory have shown that
increasing heart rate by pacing lowers stroke volume and end-diastolic diameter
has less effect on end-systolic diameter, dP/dt or flow than does isoproterenol.
the heart not only empties more completely in less time during isoproterenol
infusion, it also fills more rapidly. The more rapid filling is related to the
diastolic pressure dimension characteristics.

The normal pressure-dimension relationship during diastole is sigmoidal
in shape with slope that first decreases and then increases. Through most of
diastole, diameter and pressure increased together, but in mid-diastole pressure
declines slightly although diameter is increased. The curve is divided into

three segments: a period of elastic recoil in early diastole when pressure
is markedly negative and the slope is decreasing, a period of elastic re-
equilibrium in mid-diastole when pressure declines slightly, and a period of
elastic opposition in late diastole when the slope increases and pressure
becomes more positive. This relationship indicates that the heart exhibits
non-linear or non-Hookean elastic properties. Whether the absolute trans-
mural pressure is negative is debatable, but this fact does not alter the
general thesis of the study since changes in pressure versus diameter would
provide the same basic relationships. There is a high degree of distensi-
bility in response to relatively small stresses, a characteristic which, to-
gether with a sigmoid shape of the curves, is shared by the general class
of materials known as elastomers, which includes rubber.

Ventricular systole may be analogous to the sudden compression of an in-
flated rubber ball to a volume less than its normal unstressed volume, so
that upon release there are elastic forces which tend to return the volume
to its unstressed level. Thus, the negative pressures in early diastole
reflect this elastic tendency to return to the unstressed volume, while the
higher pressures late in diastole (the period of elastic opposition) are
due to left ventricular distention by blood forced into the chamber by
atrial contraction; this distention being opposed by the elastic forces.

The metaraminol, isoproterenol and control curves are the same general
shape but displaced from each other on both the pressure and diameter axis.
Isoproterenol induced an increased rate of force development and contraction
to a smaller end-systolic volume, findings which are compatible with increased
cardiac muscle contractility. With metaraminol induced elevation in the
arterial pressure, the heart apparently meets the challenge of the increased
aortic input impedance by shifting up its Starling curve where increased end-
diastolic fiber length results in increased total force development and
stroke volume can be preserved with less change in muscle fiber length during
ejection.

The lateral displacement of the curves is probably, at least in part,
rate related. Simple elastic elements, linear or non-linear, follow identical
stress-strain surves whether rapidly or slowly extended. However, systems in
which viscous elements are also present exhibit frequency dependency. Viscous

forces oppose distortion, so that the strain for a given stress in a system
with both viscous and elastic forces is less at higher frequencies. Thus,
if cardiac viscous elements are extant at a given pressure, increases in
heart rate or contractility will be associated with decreases in diameter.
Therefore, the shift of the curves to the left as heart rate and rate of
muscle shortening increase suggest that appreciable viscous forces are present
in the intact heart.

The slight, transient mid-diastolic decrease in pressure during the
period of elastic re-equilibration may represent stress relaxation or may
be related to change in inertial forces. Inertial forces are determined by
the mass of the mechanical system. Mass is increasing and outward acceler-
ation of the ventricular walls relatively is high in early diastole; but in
mid-diastole there is little change in mass, and acceleration is negligible.
Inertia initially opposes movement, but once the system is in motion it
actually assists the movement and would be expected to pull the pressure-
diameter curve upwards in the late stages of the early diastolic rapid fill-
ing period. Because the inertial forces are suddenly dissipated in mid-
diastole, some or all of the small decreases in pressure may be due to a
realignment of the stress-strain relationship with the elastic forces, which
are the predominant factors during a period of relatively small rate of
change. Thus, from this analysis isoproterenol may aid the heart in filling
as a result of the smaller end-systolic dimensions despite tachycardia.
During early diastole, when most filling occurs, the transmitral valve
pressure gradient is augmented, due to substantial assistance from elastic
forces which are directed toward enlarging the ventricle. On the other
hand, with metaraminol, because of the less complete systolic contraction,
there is less benefit from elastic forces in early diastole and the heart
must distend against greater elastic opposition forces in late diastole.
In the early portion of ejection these same elastic forces may aid contraction,
although this is offset by increased inertia due to the greater end-diastolic
blood mass. Since inertia initially opposes ejection of blood, the increase
dF/dt with metaraminol may be related primarily to the increased mass of
the system rather than to a decrement in myocardial contractile force.

(2)   THE INFLUENCE OF THE AUTONOMIC NERVOUS SYSTEM ON CARDIAC RESERVE AND THE
HEMODYNAMIC MECHANISMS UTILIZED IN RESPONSE TO STRESS (Vernon S. Bishop and
Lawrence D. Horwitz).

The manner in which the autonomic nervous system affects the cardiac
reserve and the hemodynamic mechanisms utilized in the response to stress
has not been extensively studied. Information has been particularly sparse
concerning the influence of autonomic activity on dynamic left ventricular
dimensions when the heart is under stress.

Utilizing the technique of Bishop (1964), an estimation of cardiac re-
serve can be obtained by determining left ventricular output curves. This
requires rapid intravenous infusions of isotonic salene until a maximum
cardiac output is reached. Cardiac performance can then be reproducibly
quantitated in a controlled laboratory setting by measuring stroke volume,
heart rate and other pertinent parameters as a function of the increased
filling pressure. To investigate the cardiac output response to alterations
in autonomic innervation during the stress of acute volume loading, left
ventricular diameter, pressure, stroke volume and heart rate were measured
in conscious dogs under conditions of normal autonomic control, beta-adrenergic
blockage, vagal blockage, and the combination of beta-adrenergic and vagal
blockage while ventricular output curves were performed. The complete
technique is described in the attached manuscript. At the time of experi-
mentation with the animals lying quietly on the dog table, beta-adrenergic
blockage was performed by administering 0.5 mg/kg to 1.0 mg/kg intravenously.
Twenty minutes after administration of the beta blocking agent propranolol,
resting measurements were obtained followed by the determination of a ven-
tricular output curves.

Acute, reversible, vagal blockage was performed by freezing the right
vago-sympathetic nerve after the left vago-sympathetic nerve had been cut.
This technique has been previously described by Stone and Bishop (1968).
A stainless steel coil was placed around the right vago-sympathetic nerve
while the animal was anesthetized with sodium pentobarbital. At the time of
the experiment, the right vago-sympathetic nerve was temporarily blocked
by infusing refrigerated alcohol at approximately 15°C.

Combination of beta-adrenergic and vagal blockage. This was performed after a series of control, beta blockage and vagal blockage studies had been completed. Twenty minutes after an intravenous infusion of propranolol, the vagus was cold blocked. Immediate resting and ventricular output measurements were made.

In the resting conscious dog, beta-adrenergic blockage with propranolol consistently increased end-diastolic and end-systolic left ventricular diameter. These increases in diameter were not due to changes in afterload or heart rate. Systemic arterial pressure was usually not altered and in some animals, particularly if the initial heart rate was slow, propranolol increased end-diastolic and end-systolic diameter without altering heart rate.

When control ventricular output curves without autonomic blockage were performed, the maximum stroke volume was attained through a substantial increment in end-diastolic diameter and a considerably smaller increment in end-systolic diameter. The maximum end-diastolic diameter with propranolol was approximately the same as that which occurs during control ventricular output curves. The reduction in maximum stroke volume was thus due solely to an increase in the end-systolic diameter. Sympathetic stimulation increases the maximum velocity of cardiac muscle preparations. Therefore, it is likely that the increases in stroke volume during acute volume loading in the presence of normal and autonomic innervation is dependent upon sympathetic discharge, whereas beta-adrenergic blockage decreases the maximum stroke volume by the extent of fiber shortening as reflected by the elevated end-systolic left ventricular diameter. At rest, vagal blockage resulted in a large increase in heart rate with simultaneous decreases in stroke volume, left ventricular end-diastolic pressure, and left ventricular end-diastolic and end-systolic diameter. At the peak of the ventricular output curves, vagal blockage did not reduce stroke volume despite the tachycardia and a significant reduction in maximum end-diastolic diameter. Although volume loading increased left ventricular filling pressure, the high heart rate with vagal blockage may have limited diastolic filling. However, with sympathetic innervation unimpaired, vagal blockage resulted in ejection of a normal stroke volume through greater cardiac muscle fiber shortening, as indicated by the reduction in peak end-systolic diameter in most animals.

When vagal and beta-adrenergic blockage were combined, resting heart rate was high, but there was not a decrease in cardiac size as occurred with vagal blockage alone. Instead, the tachycardia was accompanied by an increase in end-diastolic and end-systolic left ventricular diameter, a dimension change which resembled the resting response to beta-adrenergic blockage alone. At the peak of the ventricular output curves, the response resembled that of beta-adrenergic blockage alone in that maximum stroke volume and heart rate were usually reduced and end-systolic diameter usually slightly increased as compared with the control curves. Maximum end-diastolic diameter was significantly decreased, as occurred with vagal blockage alone.

Thus, the elimination of sympathetic innervation, whether or not vagal activity is present, impairs the stroke volume response to volume loading by decreasing the extent of cardiac muscle fiber shortening. This is reflected by greater increments in end-systolic than end-diastolic diameter during ventricular output curves performed after administration of propranolol, with or without the addition of freezing of the vago-sympathetic nerve. With sympathetic innervation intact, the ability of the cardiac muscle fibers to shorten substantially is preserved, even at high heart rates. Thus, maximum cardiac output was increased during vagal blockage because the marked augmentation of heart rate did not prevent ejection of the same maximum stroke volume as was attained during control ventricular output curves. It is difficult to understand the reduced end-diastolic diameter during volume loading with combined blockage, when the peak heart rate was less than the control level, although in excess of that with beta-adrenergic blockage alone. It is possible that absence of beta-adrenergic stimulation hampered diastolic filling at somewhat lower heart rates than was the case when autonomic innervation was intact. The relatively high resting heart rate in combined blockage may have contributed to the lack of diastolic distention of the ventricle during infusion if there was a slower rate of filling due to lack of beta-adrenergic stimulation.

(4) THE EFFECTS OF VASODILATORS ON THE CARDIOVASCULAR SYSTEM (SEE-HEMODYNAMIC EFFECTS OF NITROGLYCERIN AND AMYL NITRITE IN THE CONSCIOUS DOG) O'Rourke, Bishop, Kot and Fernandez.

The instantaneous hemodynamic effects of intravenous nitroglycerin and
amyl nitrite inhalation were compared in seven conscious mongrel dogs two
weeks after chronic instrumentation with electromagnetic flow probes around
the ascending aorta, polyvinyl catheters in the right atrium and left atrium,
ascending aorta, and left ventricular internal sonomicrometers. The decrease
in mean arterial pressure, stroke volume, and mean atrial pressures and the
increase in heart rate and cardiac output were all statistically significant,
and similar with the two drugs.

The end-diastolic diameter, the end-systolic diameter and the stroke
excursion (end-diastolic minus end-systolic diameter) decreased from control
values with both nitroglycerin and amyl nitrite.

In contrast, when the heart rate was controlled by right atrial pacing,
the decrease in end-systolic diameter and end-diastolic diameter was
accompanied by an increase in both stroke excursion and stroke volume. The
increase in stroke excursion presumably resulted from a decline in afterload,
as well as a reflex release of catecholamines. Thus, in summary, when either
nitroglycerin or amyl nitrite are given rapidly there is an early and marked
reduction in mean arterial pressure which results in a significant increase
in heart rate, myocardial contractile forces and cardiac output. There is
a decrease in atrial filling pressure due predominately to the tachycardia
but also to the increase in myocardial contractility. Decreases in left
ventricular diameter occurs for the same reason. The paced animals demonstrates
an increase of left ventricular function, independent of the Frank-Starling
mechanism, which is due to both a decrease in left ventricular afterload and
a reflex mediated increase in myocardial contractility.

(5) LEFT VENTRICULAR FUNCTION AS ASSESSED BY INCREASES IN AFTERLOAD INDUCED
BY ANGIOTENSIN (SEE-VARIABLE EFFECT OF ANGIOTENSIN INFUSION ON LEFT VENTRICULAR
FUNCTION (O'Rourke, Pegram and Bishop).

The effects of increase in afterload on left ventricular function by the
infusion of angiotensin was of interest from two standpoints. First, the
response of chronic dogs to artificially induced afterload, produced by partial
aortic obstruction which is probably not physiological, provided us with in-
consistent responses in conscious animal studies. Secondly, left ventricular

function has been assessed routinely in both experimental animals and man by the use of increases in afterload induced by angiotensin infusion. In six chronically instrumented animals with aortic flowmeters, left ventricular internal sonomicrometers and catheters in the right and left atrium, angiotensin infusion caused significant increases in left ventricular mean systolic pressure. At the peak effect the average left ventricular mean systolic pressure changed from 84 to 120 mmHg, left ventricular end-diastolic pressure increased from 4 to 23 mmHg, and cardiac output decreased from about 1.9 liters to 1.2 liters, and while stroke volume decreased from an average of 16 cc/beat to 11 cc/beat. All these changes were significant.

With increases in afterload, the end-diastolic and end-systolic diameters increase while the extent of shortening and stroke volume decreased. Ventricular function curves were obtained by plotting the stroke volume or stroke work as a function of the increasing end-diastolic pressure. The stroke work curve or stroke volume curves were variable between each animal and among the group. At any given end-diastolic pressure there was a variable stroke volume or stroke work response. In any given animal it was impossible to tell on successive determinations whether the left ventricular function was good or bad.

We concluded from this study that a continuous graded intravenous infusion of angiotensin produces an increase in left ventricular mean systolic and end-diastolic pressures, an increase in end-diastolic diameter and a greater increase in end-systolic diameter. This is associated with a decrease in cardiac output, stroke volume and stroke excursion and no significant change in the heart rate. Ventricular function curves obtained from this data vary significantly when compared in similar animals of the same species and may differ considerably in the same conscious animal on different determinations. These data suggest that ventricular function curves obtained by this method provide an unreliable index of ventricular function. It is apparent that the effects of afterload on left ventricular performance is not a simple effect. Angiotensin-induced increases in afterload at various heart rates and filling pressures are presently being evaluated as a means to quantitate the relationship of these three variables on left ventricular function.

(6) LEFT VENTRICULAR INTERNAL DIAMETER OBTAINED BY CARDIAC CATHETERIZATION (SEE-MEASUREMENT OF LEFT VENTRICULAR INTERNAL DIAMETER BY CATHETERIZATION) Kardon, O'Rourke, Palmer and Bishop.

Our studies with chronic instrumented conscious animals have provided important information regarding the interrelationship of left ventricular internal

diameter, left ventricular pressure and stroke volume. These studies have emphasized the need for continuous measurements of left ventricular internal diameter and pressure by procedures which would not require a thoracotomy and could be readily used in research animals and man.

The lack of a suitable technique has led to our development of a catheter which measures the internal diameter and pressure of the left ventricle. The basic principle involves the measurement of the mean transient time for ultrasound to traverse the distance between piezoelectric crystals mounted at appropriate distance along a number 8F woven dacron catheter. The catheter is preformed and is passed retrograde up the femoral artery and into the left ventricle, and positioned so that a semicircular loop of the catheter lies in a plane parallel to the septum and against the anterior and posterior endocardial surface. In this position one piezoelectric crystal is on the posterior endocardial surface. Usually, the loop of the catheter traverses the major cord of the left ventricle parallel to the interventricular spetum, and, in fact, the catheter exhibited a tendency to seek the largest dimension. Because the transducers are radiopaque, it is always easy to determine the exact position and the plane of orientation.

The left ventricular transverse internal diameter recording obtained with the dimension catheter in six anesthetized animals under resting conditions and with angiotensin and isoproterenol infusions were similar to the internal diameter response obtained from animals which had been chronically instrumented with sonomicrometers. This catheter, so far, has demonstrated a unique way of evaluating the left ventricular internal dimensions of the heart on a beat-to-beat basis. In addition to the general advantage of the ultrasound technique, the piezoelectric crystals are radiopaque and thus allow for easy visualization of the plane of measurement and the movement of the piezoelectric crystal against the anterior and posterior endocardial surface. In all animals studied the number of premature ventricular contractions occurring during the positioning of this catheter were similar to those obtained during routine catheterization of the left ventricle and were transient. Thus, we believe this technique should be extremely valuable in the assessment of left ventricular function in non-thoracotomized animals and with refinement may be of value in diagnostic left heart catheterization in patients with heart disease.

(7)   THE ROLE OF ACETYLCHOLINE AND NOREPINEPHRINE IN CONTROLLING HEART RATE
(SEE-THE INTERACTION OF ACETYLCHOLINE AND NOREPINEPHRINE ON HEART RATE) G.O.
Carrier and V.S. Bishop.

In conscious animal studies one often uses the resting heart rate to eval-
uate the degree of excitability of the animal. The heart rate may be high
on certain days and lower on other days and yet in both cases the animal may
be resting quietly, even dozing. In responses to stress one often states,
based on heart rate changes, that the stress causes an increase in sympathetic
activity or an increase in vagal activity. The purpose of our study was to
investigate the interaction between acetylcholine and norepinephrine on a
chronotropic response in isolated rabbit atria so that a quantitative relation-
ship between the two neurotransmitters could be established. Recently,
Grovner et al, 1970, described the interaction of acetylcholine and norepine-
phrine on isolated rabbit atria. The results obtained by these investigators
were qualitatively in agreement with our findings (Carrier and Bishop, 1970).
However, the concentration of acetylcholine employed by Grovener was 100
times the concentration of norepinephrine. This difference in concentration
could be considered to bias the results in favor of acetylcholine effect.

In our study the data clearly indicates the vagus transmitter, acetyl-
choline, has a greater affinity for the mechanism responsible for alterations
in heart rate than does norepinephrine when both transmitters are present.
When the isolated atria are subjected to acetylcholine or norepinephrine
separately, a slowing or acceleration in heart rate respectively occurred.
In the presence of 10^{-8}M acetylcholine, norepinephrine concentrations caused
a significant increase in heart rate. At this concentration of acetylcholine,
we can assume that there was virtually no cholinergic influence present. There-
fore, one would expect to see a pure adrenergic response. Increases in the
concentrations of acetylcholine to 10^{-7}M resulted in significantly higher
doses of norepinephrine being required to obtain maximum responses or reversed
the slowing effects of acetylcholine. In the presence of 10^{-5}M acetylcholine,
which causes approximately 60% depression in rate, a concentration of nor-
epinephrine at least 100 fold greater than that of acetylcholine is required
to cause a slight reversal of acetylcholine's effect.

The data suggest that competitive interaction between the neurotransmitters
effect the changes in heart rate, and that acetylcholine apparently has a

greater effect on the heart rate slowing for equimolar concentration than that
of norepinephrine. These results suggest that the parasympathetic and
sympathetic system do not have to act in any reciprocal fashion, nor do they
add in an algebraic fashion. Thus, the resting heart rate may be set by the
vagus and modulated by changes in sympathetic activity.

STUDIES IN PROGRESS

(1) Effects of Atrial Pacing

Generally as the heart rate increased, both the end-diastolic diameter
(EDD) and the end-systolic diameter (ESD) decreased. The change in EDD was
usually two to three times the change in ESD. At the highest atrial pacing
(d=+117) the ESD was increasing back toward the control values even though
the left ventricular mean systolic pressure was not elevated. The correspond-
ing reduction in stroke volume was the result of a reduced stroke diameter
occurred as a result of a diminished initial diameter (EDD) and the lack of
a corresponding reduction in ESD.

As has been shown previously by this investigator and by others, the
stroke volume was found to decrease linearly with increases in heart rate
in all animals studied. However, many factors may alter this relationship
such as changes in initial filling pressure, aortic pressure, and the inotro-
pic state of the heart. Furthermore, the relationship is time dependent
and inhibits a hysteresis when the heart rate is reduced from the high rate
back to the initial rate. This is probably related to the changes in filling
pressure since both the mean atrial pressure and the internal diameter exhibit
similar hysteresis. The effects of the initial mean left atrial pressure or
end-diastolic pressure, inotropic state and aortic pressure on the stroke volu..
heart rate relationship is presently being investigated. The average mean
left atrial pressure was not significantly reduced until high heart rates were
reached. It usually declined in an asymptotic relationship to the heart rate.

The left ventricular transverse internal diameter changes rapidly during
ejection and during the phase of rapid filling. With exception of two animals
the absolute value of the first derivative with respect to time (dD/dt) of the
diameter was greater during the rapid filling phase dDd/dt than during the
ejection phase dDs/dt. This means the circumferential fiber lengthening rate
was greater than the shortening rate. As the heart rate is increased, the rapid
filling phase fused with the slow filling phase and dDd/dt increased slightly
with moderate increases in heart rate. The derivative of the diameter during
ejection dDs/dt decreased only at very high heart rates. More work is being

conducted with respect to the factors effecting the velocity of lengthening
during the heart cycle. Since the harmonic components of the diameter
measurement are relatively low frequency any artifact in the measurement
may significantly effect the derivative.

Pacing had little effect on left ventricular mean systolic pressure or on
dP/dt (max). A reduction of about 10% was seen only at the highest rates.
The acceleration of the blood during ejection (dF/dt) decreased with all
elevated heart rates reaching a 25% reduction at the highest rate. From
physical consideration, dF/dt must depend upon the initial diameter (EDD),
the extent of muscle shortening (EDD-ESD) and the rate of muscle shortening.
Thus, the dF/dt would be expected to decrease with atrial pacing if the ino-
tropic state of the heart was not greatly altered.

Normally, atrial pacing exerts some positive inotropic effect on the
left ventricle which is reflected by an increase dP/dt (max). However, the
EDD and the extent of shortening also have an effect on dP/dt (max). Thus,
the reduction in the EDD and the extent of shortening may offset any in-
crease in dP/dt (max) which may result from the atrial pacing. In preliminary
experiments, removal of the sympathetic input to the heart, the stroke volume
decreased as usual with increasing heart rate but ESD, instead of decreasing,
now increased. Additional experiments are being undertaken to investigate
the mechanism of this response.

(2) Effects of Afterload

In the conscious dog graded changes in afterload were difficult
to obtain using the technique of occluding aortic flow. An elevation of the
mean arterial pressure (MAP) by 17 mmHg, which caused the left ventricular
mean systolic pressure to increase 21 mmHg, resulted in significant change in
mean left atrial pressure (MLAP), heart rate, dF/dt (max), EDD and ESD. With
larger afterload imposed upon the left ventricle tension which has to be
developed by the left ventricular muscle is increased. Since both left ven-
tricular pressure and diameter are increased, the afterload as reflected by
the changes in either MAP or LVMSP is less than the increased developed tension.

The maintenance of the stroke volume during the elevated afterload was
due to an increase in MAP and EDD. Since the ESD was increased, the increased
EDD resulted in a maintenance of the extent of shortening. It is unlikely that

the inotropic state of the left ventricle was changed since dP/dt and the extent shortening were not increased.

The acceleration of the blood dF/dt (max) from the left ventricle was diminished in face of the increased afterload. The reduction in the rate of the diameter change during ejection (dDs/dt) and rapid filling (dDd/dt) were not significant. These changes may be related in part to the decrease in heart rate.

(3) Effects of Afterload and Atrial Pacing

The effects are similar to the effects seen during atrial pacing alone. Many of the changes are not significant.

The effects of afterload plus pacing are compared to the resting control state. Mean left atrial pressure and left ventricular mean systolic pressure were still elevated above control. The acceleration of blood while reduced with afterload alone is now increased about 10%. The stroke volume decreased principally because the ESD was maintained above the control value. The reduction in the extent of shortening was similar to that seen with atrial pacing alone because the EDD was similar to the control resting value and ESD was elevated.

(4) Critique of Atrial Pacing and Afterload Changes

The techniques used for increasing the heart rate and afterload were not satisfactory for routine use in conscious animals. Stable reproducible levels of atrial pacing were often difficult and pacing artifacts were often present in the electromagnetic flow signal. The artificially induced afterload was difficult to obtain in a reproducible manner. Furthermore, as mentioned previously, the degree of aortic occlusion was not directly related to the change in mean arterial pressure. Often times due to the discomfort displayed by the animals, a significant elevation in mean arterial pressure could not be obtained.

As a result of the above factors this portion of the research project has been changed as follows:

(1) Increases in heart rate are obtained by either:

    a. Atrial pacing by previously implanted electrodes on the right atrium.

    b. Blockage of the vagus by atropine or cold blocked

(2) Increases in afterload are obtained by infusing

    a. Angiotensin or..

    b. Phenylephrine

(5) Mathematical Description of Ventricular Output Curves

Generally, the ventricular output curves determined by acutely
preloading a conscious animal at a constant rate can be described by the
following equation: $dc/dp = K (Cm-Co)$ where.....C = cardiac output (cc/min)
.....K = proportionally constant $mmHg^{-1}$ .... Cm = maximum cardiac output
..........Co = initial cardiac output, or cardiac output at any pressure.
Based upon the heart lung preparations one would expect a first order equation
to be more applicable to the stroke volume response. In conscious dogs the
heart rate response to increases in preload are often variable and thus the
stroke volume. We are now evaluating this response in terms of the stroke
volume by maintaining the heart rate constant by vagal blockage. In the above
equation, cardiac output can then be replaced by stroke volume (S). Example:
$ds/dp = K (Sm-So)$.

With heart rate fixed this equation describes the stroke volume response
based upon the Frank-Starling principle. The equation states that change in
stroke volume per increment change inffilling pressure is proportional to the
stroke volume reserve of the left ventricle. The Frank-Starling principle
would imply that ds/dp would be large at first and then decrease as the stroke
volume increased. Thus, based upon this premise, we have investigated the
stroke volume output response in conscious animals instrumented with electro-
magnetic flowmeters and catheters for rapid infusion of Tyrode's solution and
measurement of left atrial pressure. Ventricular output curves and stroke
volume output curves have been obtained under control states, vagal blockages
with atropine and with atropine plus isoproterenol. The dose of isoproterenol
average 0.1 g/kg and was purposely maintained at low levels so not to in-
fluence the heart rate response to atropine alone. The cardiac output reserve
(Cm-Co) was increased from a control value of 102 cc/kg-min to 140 cc/kg-min
and 160 respectively for atropine and atropine plus isoproterenol treated
animals. With atropine the stroke volume reserve increased over the control
principally as a result of the lower initial stroke volume (So) and the higher
heart rate. However, the maximum stroke volume (Sm) at the peak of the output
curve was not different. Comparing the atropine to atropine plus isoproterenol
the initial slope ds/dp was 0.14 cc/kg-beat for atropine treated and 0.22 cc/kg-
beat for the atropine plus isoproterenol treated animals. Isoproterenol also
increased the sttoke volume reserve (Sm-So) from 0.60 cc/kg/min to 0.90 cc/kg-min.

Thus, the initial study has demonstrated that by maintaining the heart rate constant one can evaluate the pumping ability of the heart in terms of stroke volume and that small inotropic interventions can be easily detected by mathematically analyzing the data. Studies are now underway to incorporate the internal diameter responses in this treatment so that stroke volume will be related to the diameter changes.

(6) Pericardium

In evaluating the role of the pericardial sac in the performance of the heart in health and disease, we have continued to look at the permeability of the membrane. In the rabbit, dog, and human pericardium, we have found the membrane not only to have a high filtration coefficient (the measurement of the amount of fluid which can pass across the membrane per cm H_2O pressure), but that the permeability of the membrane to creatine and urea are 1.5×10^2-3 cm/sec and 3.2×10^{-5} cm/sec when molecules of the size of glucose are studied. Although the exact function of the pericardium is not known, we believe these characteristics of the membrane must be considered important functions of the pericardium in the regulation of the fluid contained between the pericardium and the heart.

(7) Evaluation of the Interaction of Propranolol and Digitalis

One of the earlier clinical applications of beta-adrenergic blocking (propranolol) agents was the treatment of patients with angina pectoris. This blockage of the sympathetic nerves has been shown by us to reduce left ventricular function, (both the EDD and ESD are increased at rest). This becomes more important when considering the response to stress. Since this drug (propranolol) is widely used and its depressive action could precipitate heart failure in these patients, we have investigated to see if digitalis, which is often given with propranolol, would reverse the depression resulting from propranolol alone. In general when propranolol is given to resting, animals, digitalis negates the depressive action of propranolol. When digitalis is given first, the opposite is true. Thus, at rest digitalis may prevent a patient from developing severe heart depression from propranolol. This may or may not be true during exercise.

(8) Force Velocity

In all studies in which left ventricular internal diameter and pressure have been measured and in which we have evaluated the response of the left ventricle,

we have calculated velocity of shortening of the contractile element and the
series elastic element as well as the tension. These calculations have required
major assumptions which is true of all velocity measurements made in the intact
heart. Our direct diameter measurements usually indicate shortening (Horwitz
and Bishop) during isovolumic contraction. This alters the force-velocity
relationship during the most important period. We are presently making a de-
tailed analysis of all the force-velocity relationships we have obtained in
some twenty animals under various experimental conditions. We are also in-
vestigating the tension change, the impulse and momentum of the muscle and blood.
So far out most reliable index of myocardial contractility is the extent of
shortening and the end-systolic diameter.

LEFT VENTRICULAR PRESSURE-DIMENSION

RELATIONSHIPS IN THE CONSCIOUS DOG

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Running Head: Left Ventricular Pressure-Dimension

Relationships

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ABSTRACT

The dynamic function of the left ventricle was described in
terms of internal diameter, pressure and flow in seven conscious,
unsedated dogs at rest and during isoproteronol and metaraminol
infusion. Diameter was approximately linearly related to left ven-
tricular volume during ejection. Isoproteronol was associated with
increases in heart rate, left ventricular systolic pressure, dP/dt,
dF/dt, and dD/dt, and decrease in end systolic and end diastolic
pressure and diameter. Metaraminol induced increases in left ven-
tricular systolic and diastolic pressures and diameters and decreases
in heart rate and dF/dt. Stroke volume was not affected by either
drug. During diastole the pressure-diameter relationship was sigmoidal
with the initial portion markedly negative in pressure. Changes in
heart rate, contractile state, and afterload resulted in displacement
of the diastolic curves. It is concluded that viscous forces, and
possibly inertial forces, influence the diastolic stress-strain re-
lationship, and that elastic recoil is present in early diastole in
the normal heart.

Key Words: Left Ventricular Diameter, Maximum Acceleration, Stroke
            Work, Left Ventricular Compliance, Viscoelasticity, Inertia
            of the Heart, Stress Relaxation.

Accurate, quantitative descriptions of dynamic left ventricular dimensions have been difficult to obtain, particularly in conscious animals. Volume measurements with cardiometers fail to distinguish between the right and left ventricles, while roentgenographic techniques are limited in resolution, require geometric approximations and are liable to unphysiological alterations from dye injections (9,15). Electronic gauges affixed to the external surface of the heart are unsatisfactory for assessment of ventricular chamber size because wall thickness changes, which may be substantial (4), cannot be detected.

Recently, continuous instantaneous measurements of the internal transverse left ventricular diameter have been recorded with ultrasonic transducers chronically affixed to the endocardium of conscious dogs (6). Changes in this dimension were approximately linearly related to changes in ventricular volume during ejection, indication that internal diameter is a reliable index to ventricular volume (1).

The purpose of this study is to describe the dynamic function of the left ventricle in terms of its diameter, pressure and outflow at rest and during infusions of isoproteronol and metaraminol in conscious, unsedated dogs. Special attention was focused on the nature of the diastolic stress-strain pattern of the ventricle and its influence on filling.

METHODS

Thoracotomies were performed in eight 18-27 kg mongrel dogs anesthetized with methoxyflurane. During a brief venous inflow occlusion,

a stab incision was made th. ough the anterior wall of the left ventricle and two discoid piezoelectric crystal transducers, 4 mm in diameter and 2 mm in thickness, were implanted within the chamber (6). The transducers were positioned across the greatest internal transverse left ventricular diameter, one on the anterior and the other on the posterior endocardial wall. Through a second incision on the anterior wall, approximately 2 cm above the apex, a solid state pressure transducer (Microsystems--now Whittaker Corporation #1017) was also implanted within the left ventricle. An electromagnetic flow probe was placed around the ascending aorta and a polyvinyl catheter inserted into the left atrium through the left atrial appendage. The pericardium was left open. The catheter and wires were brought outside the skin at the back of the neck. The dogs were given two weeks for recovery from surgery before experiments began; none had arrhythmias or systemic infections and all could exercise normally. During recordings, the animals lay on their right sides, unsedated, but lightly restrained.

A sonomicrometer designed by Stegall et al. (17) measured transit time of 5 mHz ultrasound between the two piezoelectric crystals at a sampling rate of 5000 times per second. Since the velocity of sound in blood is known, transit time was convertible to distance. Flow signals were recorded with a Medicon K 2000 electromagnetic flowmeter. The late diastolic level of aortic flow was used as the zero flow reference point. Flow probes were calibrated in vitro prior to implantation, and the calibration verified at autopsy where in all cases

the two calibrations varied by less than 5%.

Left atrial pressure was measured through the implanted catheter with a Statham P23db manometer zeroed to the midline of the sternum while the dog lay on its right side. The solid state pressure transducers were precalibrated at $38^{\circ}C$ and sensitivity did not change during implantation, although some drift in the zero level did occur from day to day. Zero ventricular pressure was checked on some occasions with a catheter inserted percutaneously into the left ventricle and an external manometer, but since this sometimes perturbed the animal, the zero drift was corrected in most records by setting the left ventricular end diastolic pressure equal to the left atrial pressure at the beginning of each experiment. In one animal, sedated with Innovar (McNeil Laboratories), intrapleural pressure was estimated with a catheter in the esophagus and an external manometer during rest, isoproterenol infusion and metaraminol infusion. The electrocardiogram was obtained with subcutaneous needle electrodes placed over the sternum.

All signals were inscribed on an Offner Type R oscillograph and an Ampex FR 1800 tape recorder. The taped records were processed with analogue and digital computers. The digital program averaged ten consecutive beats and was triggered by the R wave of the electrocardiogram. Recordings of left ventricular pressure and diameter and phasic and integral aortic flow was made every .008 seconds. Single beats were also analyzed.

Measurements of resting values were followed by intravenous infusion of either isoproterenol hydrochloride at a rate sufficient to

increase heart rate by approximately 25% or metaraminol bitartrate
at a rate sufficient to elevate mean aortic pressure by approximately
30%. Usually, both infusions were given the same day; some dogs re-
ceived only one of the drugs.

CRITIQUE OF METHODS

The solid state pressure gauges were not tested for frequency
response but based on their electronic characteristics and reports
by others they should have a natural frequency in excess of 3000 cps
(10). Changes in sensitivity _in vitro_ and _in vivo_ appeared to be
negligible. Significant zero drift during implantation did occur from
day to day, however, and an independent zero reference was needed to
set the pressure at the beginning of each experiment. Catheters tem-
porarily inserted into the left ventricle under local anesthesia were
on occasion used for such a reference, although the catheter left
ventricular pressure, which was matched with the solid state transducer
pressure, was subject to some error because of relatively low fre-
quency response and motion artifacts. The catheter left ventricular
end diastolic pressures were always within 1 mm Hg of the mean left
atrial pressure whether control, metaraminol or isoproteronol states
were studied. This correlation was judged sufficiently good to justify
setting the solid state transducer ventricular end diastolic pressure
equal to the mean atrial pressure on a day to day basis. Braunwald
and Frahm have reported that left atrial pressure correlates within
0.2 mm Hg with left ventricular end diastolic pressure in normal man
(2). This was preferred to repeated catheterization of the ventricle,

which would not permit good resting states. Errors from this method could have affected the absolute levels of left ventricular pressure by as much as 1 mm Hg, but relative values during a cycle were correct.

The diameter measurement was of very high frequency response and resolution. The sonomicrometer has a theoretical resolution of 1/4 wavelength which corresponds to .07 mm at 5 mHz. Frequency response is a function of the sampling rate of 5000 times per second.

Flow probe calibrations were rechecked and found to be unchanged after the animals were sacrificed, and errors from the calibration or from inaccuracy in the assumption that end diastolic flow is zero were probably slight. Both the sonomicrometer and the flowmeter were found to have phase lags of approximately 40° at 20 Hz. The lag in the pressure record may be assumed to be negligible. Therefore, diameter and flow could be compared in time and the phase lag in these measurements was so small that, using the .008 second interval for the readings, they could also be compared with pressure, since the estimated delay was less than a single interval.

Results were similar whether individual or averaged beats were used. Averaged beats were used for the data presented, because extraneous electrical noise, motion artifacts, respiratory effects and single abnormal beats could be averaged out to insure greater accuracy.

RESULTS

Figure ' is a photograph of a digital computer plot of left ventricular diameter, pressure, phasic aortic flow, and integral aortic flow versus time. Ten consecutive beats in a resting animal are

averaged. At the onset of systole, left ventricular pressure rose abruptly, while simultaneously the left ventricular diameter decreased. An average of 11% of the total decrease in diameter occurred before there was any flow through the aortic transducer. During ejection, diameter continued to decrease, with the most rapid rate of change occurring simultaneously with the peak flow. Diameter then increased, rapidly at first, slowly in mid-diastole, and again rapidly late in diastole at the time of atrial contraction.

Figure 2 from the same record plots diameter versus integral flow and pressure. In every animal diameter and flow were approximately linearly related in all states studied. Since over a relatively small range it is difficult to distinguish between linear, square or higher power functions, a statistical analysis of the diameter versus flow curve was performed with a digital computer to determine the nature of the relationship. In all cases a linear function described the relationship to a high degree of statistical significance. Although addition of a square or cubic term improved the fit in some curves, the linear component was always the major factor. A linear function also closely described the diameter--flow relationship during rapid intravenous infusions in a previous study (1).

In the resting animal, the pressure versus diameter plot formed a parallelogram-like loop with the beginning of systole at the lower right hand corner During the initial phase of systole, a large increase in pressure was accompanied by a small decrease in diameter. During most of ejection, pressure was unchanged while diameter de-

creased markedly. Late in systole, pressure fell rapidly, while diameter was only slightly altered. During diastole, a small rise in pressure was accompanied by a large increase in diameter.

Figure 3 superimposes the plots of each of the ten beats averaged in the previous two figures. Beat-to-beat variations were remarkably small despite a slight sinus arrhythmia.

Figure 4 graphs the derivatives of diameter, pressure, and flow along with the instantaneous values of each. Diameter changes are more rapid during early diastole than during ejection. This more rapid rate of change of diameter during filling was present in all states and was particularly marked during isuprel infusions.

Tables 1 and 2 summarize the hemodynamic changes during isoproteronol infusions in six dogs and metaraminol infusions in seven dogs. Stroke volume did not change during administration of either drug. Isoproteronol was associated with statistically significant increases in heart rate, left ventricular systolic pressure, maximum rate of pressure rise, maximum acceleration of blood during ejection, and maximum rate of decrease in diameter, and decreases in end systolic and end diastolic diameter and left ventricular end diastolic pressure. Metaraminol was associated with increases in left ventricular systolic and diastolic pressures and end systolic and end diastolic diameters; heart rate and acceleration decreased. Metaraminol did not affect maximum rate of rise of pressure or maximum rate of decrease in diameter.

Figure 5 plots, from a single record, pressure versus diameter during the diastolic filling period, starting when pressure is at its

minimum value and ending just before systole. The curve is sigmoidal in shape with a slope that first decreases and then increases. Through most of diastole diameter and pressure increase together, but in mid-diastole pressure declines slightly, although diameter is increasing. The curve is divided into three segments: a period of elastic recoil in early diastole, when pressure is markedly negative and the slope is decreasing, a period of elastic reequilibration in mid-diastole, when pressure declines slightly, and a period of elastic opposition in late diastole, when the slope increases and pressure becomes positive.

The upper half of Figure 6 shows the diastolic pressure-diameter relationship for control, isoproteronol and metaraminol states in another dog. In most animals, isoproteronol curves were to the left of and began at more negative values than the control, while metaraminol curves were to the right of and began at less negative values than the control. As shown in the bottom half of Figure 6, when isoproteronol was infused at different rates the curve representing the faster heart rate was shifted to the left. When heart rate varied by less than 10 beats/min isoproteronol curves were nearly superimposed.

Mean intrapleural pressure at rest was -2 mm Hg in the single animal in which it was measured. There was no change in mean intrapleural pressure during isoproteronol or metaraminol infusion.

DISCUSSION

Studies with a variety of techniques have confirmed that the left ventricle ejects blood primarily by shortening in the transverse dimension and that changes in the apex-to-base dimension are slight (5,13). In this study the relationship between volume ejected and

left ventricular internal transverse diameter was essentially linear
during control, isoproteronol and metaraminol states in all animals.
In a previous study (1), a similar relationship was demonstrated dur-
ing volume loading by rapid intravenous infusion of Tyrode's solution.
Because the linear relationship is present over a wide range of stroke
volumes and heart rates, this single dimension measurement is a reason-
ably accurate index of left ventricular volume changes during ejection.

During the so-called "isovolumic" portion of systole, when
pressure is rising but has not yet reached the aortic level, diameter
decreased by an average of 11% in control and metaraminol states,
slightly less during isoproteronol, yet no flow occurred through the
flow probe on the ascending aorta. Pieper, using a catheter tip di-
mension gauge, has also detected a decrease in internal diameter during
the isovolumic period (11). Other investigators have reported increases
in external diameter or circumference (5,14), but these measurements
are influenced by wall thickening during the isovolumic period (4).
Possible causes of the internal diameter change prior to ejection are
accumulation of blood in the mitral valve region, where the highly
elastic valves may bulge toward the atrium as the ventricular pressure
exceeds the atrial pressure, or a segmental contraction pattern with
portions of the ventricle contracting before others (12).

Isoproteronol induced tachycardia, a decrease in left ventricular
end diastolic and end systolic diameters, and a decrease in left ven-
tricular end diastolic pressure; metaraminol induced bradycardia, in-
crease in end systolic and end diastolic diameters and an increase in

end diastolic pressure. Neither drug appreciably altered stroke
volume in most dogs. DP/dt and circumferential shortening rate,
measurements commonly cited as indicative of myocardial contractile
strength, increased with isoproteronol and were unaffected by
metaraminol. Another measurement regarded by some investigators as
an index of contractility, dF/dt did not change significantly with
isoproteronol and decreased with metaraminol.

It would appear that the heart counteracts an increase in
afterload by increasing end diastolic size whereas beta adrenergic
stimulation is characterized by production of the same stroke volume
from an end diastolic volume less than that of the control by means
of a more complete contraction and a smaller end systolic volume.
Whether one approximates a sphere or any prolate spheroid to the left
ventricle, to produce the same change in volume requires a smaller
change in diameter if the initial volume is large than if it is small.
Therefore, as would be expected, the change in diameter during ejection
is less than the control during metaraminol infusion but exceeds the
control during isoproteronol infusion.

The bradycardia of metaraminol is presumably a reflex mechanism
under baroreceptor control and secondary to the rise in pressure,
whereas the tachycardia of isoproteronol is due to beta adrenergic
stimulation of the sino-atrial node. The effects of tachycardia alone
do not account for the changes noted with isoproteronol. Unpublished
data in our laboratory, which correlates with numerous other studies,
indicates that increasing heart rate by pacing lowers stroke volume and

end diastolic diameter but has little effect on end systolic diameter,
and has less effect on rates of change of pressure, flow or diameter
than does isoproteronol. The heart not only empties more completely
in less time during isoproteronol infusion; it also fills more rapidly.
The more rapid filling is related to the diastolic pressure-dimension
characteristics of the heart.

The pressure-dimension curves in Figure 5 indicate that the heart
exhibits nonlinear or non-Hookean elastic properties. There is a high
degree of distensibility in response to relatively small stresses, a
characteristic which, together with the sigmoid shape of the curves,
is shared by the general class of materials known as elastomers, which
includes rubber. Similar, sigmoid shaped, stress-strain curves have
been reported in static measurements of fibrillating hearts and other
hollow organs such as urinary bladder (7,8).

The initial portion of the curve, described in Figure 5 as the
period of elastic recoil, is negative in pressure. Ventricular systole
may be analogous to the sudden compression of an inflated rubber ball
to a volume less than its normal unstressed volume, so that upon release
there are elastic forces which tend to return the volume to its un-
stressed level. Thus the negative pressures in early diastole reflect
this elastic tendency to return to the unstressed volume while the
higher pressures late in diastole, the period of elastic opposition, are
due to left ventricular distention by blood forced into the chamber by
atrial contraction, this distention being opposed by the elastic forces.

The stress actually exerted on the left ventricle during diastole
is not the intraventricular pressure but rather the transmural pressure.

Since, as in the one animal where a measurement was made, the mean intrapleural pressure is usually slightly negative, the true transmural pressure is higher at any instant by perhaps one or two mm Hg than the measured intraventricular pressure. Data is taken from beats averaged over several respiratory cycles; therefore, only the mean intrapleural pressure is a factor and instantaneous changes in pleural pressure do not influence the results. Neither isoproteronol nor metaraminol appreciably altered mean intrapleural pressure in the dog with the intraesopageal catheter, and differences in diastolic stress-strain patterns during infusions of these drugs cannot be accounted for by variations in depth of respiration. The existence of a lower pressure inside than outside the ventricle during early diastole is indisputable, since the effects of small variations in zero levels or intrapleural pressures are insufficient to entirely account for the negativity of the intraventricular pressures. Negative diastolic ventricular pressures have been postulated by others (16).

The metaraminol, isoproteronol and control curves are of the same general shape but displaced from each other on both the pressure and diameter axes. Isoproteronol induced an increased rate of force development, and contraction to a smaller end systolic volume, findings compatible with increased cardiac muscle contractility. With metaraminol-induced elevation in the arterial pressure, the heart apparently meets the challenge of the increased aortic input impedance by shifting up its Starling curve to where increased end diastolic fiber length results in increased total force development and stroke volume can be

preserved with less change in muscle fiber length during ejection.

The lateral displacement of the curves is probably, at least in part, rate related. Simple elastic elements, linear or nonlinear, follow identical stress-strain curves whether rapidly or slowly extended. However, systems in which viscous elements are also present exhibit frequency dependence (16). Viscous forces oppose distortion, so that the strain for a given stress in a system with both viscous and elastic forces is less at higher frequencies. Thus, if cardiac viscous elements are extant, at a given pressure increases in heart rate will be associated with decreases in diameter. Therefore, the shift of the curves to the left as heart rate and rate of muscle shortening increase suggest that appreciable viscous forces are present in the intact heart.

The slight, transient mid-diastolic decrease in pressure, during the period of elastic reequilibration, may represent stress relaxation or may be related to change in inertial forces. Inertial forces are determined by the mass of the mechanical system. Mass is increasing, and outward acceleration of the ventricular walls relatively high, in early diastole, but in mid-diastole there is little change in mass and acceleration is negligible. Inertia initially opposes movement, but once the system is in motion it actually assists the movement and would be expected to pull the pressure-diameter curve upwards in the late stages of the early diastolic rapid filling period. Because the inertial forces are suddenly dissipated in mid-diastole, some or all of the small decrease in pressure may be due to a realignment of the stress-strain relationship with the elastic forces, which are the predominant factors during a period of relatively small rate of change.

The smaller end systolic dimension during isoproteronol infusion
is advantageous to the heart in filling adequately, despite tachy-
cardia. During early diastole, when most filling occurs, the trans-
mitral valve pressure gradient is augmented, due to substantial
assistance from elastic forces which are directed toward enlarging
the ventricle. With metaraminol, because of the less complete systolic
contraction, there is less benefit from elastic forces in early diastole
and the heart must distend against greater elastic opposition forces
in late diastole. However, in the early portion of ejection these same
elastic forces aid contraction, although this is offset by increased
inertia due to the greater end-diastolic blood mass. Indeed, since
inertia initially opposes ejection of blood, the decreased dF/dt with
metaraminol may be re'ated primarily to the increased mass of the
system rather than to a decrement in myocardial contractile force.

TABLE 1. Hemodynamic effects of isoproterenol.

		HR beats/min	SV cc/beat	LVSP mm Hg	LVEDP mm Hg	EDD mm	ESD mm	MAX dP/dt mm Hg/sec	MAX dF/dt cc/sec²	MAX dD/dt cm/sec
Control	X̄	81	21.0	134.0	2.9	36.3	28.7	3048	5255	6.1
	SD	19	6.5	29.3	2.6	3.1	3.5	554	1455	1.0
Isoproterenol	X̄	112	19.9	141.9	0.8	34.9	27.0	3977	7717	7.4
	SD	18	5.4	28.0	1.9	3.4	3.8	943	1667	1.5
P		<.01	N.S.	<.05	<.05	<.05	<.005	<.005	N.S.	<.05

HR = heart rate, SV = stroke volume, LVSP = left ventricular systolic pressure, LVEDP = left ventricular end diastolic pressure, EDD = end diastolic diameter, ESD = end systolic diameter, MAX dP/dt = maximum rate of change of left ventricular pressure, MAX dF/dt = maximum acceleration of blood, MAX-dD/dt = maximum rate of decrease in left ventricular diameter.

P values obtained by paired sample comparisons of per cent changes.

Degrees of freedom = 5. NS = Not Significant (P <.05), X̄ = mean, SD = Standard deviation.

TABLE 2. Hemodynamic effects of metaraminol.

		HR beats/min	SV cc	LVSP mm Hg	LVEDP mm Hg	EDD mm	ESD mm	MAX dP/dt mm Hg/sec	MAX dF/dt cc/sec²	MAX dD/dt cc/sec
Control	X̄	75	22.7	121.7	3.0	35.4	27.9	2625	6331	6.3
	SD	15	7.0	20.8	2.4	3.9	4.4	446	1338	0.7
Metaraminol	X̄	63	21.1	153.3	8.2	36.6	29.0	2947	5534	5.8
	SD	8	4.4	29.4	2.7	3.4	4.2	820	1141	1.2
P		<.05	N.S.	<.001	<.01	<.01	<.05	N.S.	<.005	N.S.

Sees symbols and statistical analysis as Table 1. Degrees of freedom = 6.

LEGENDS

Figure 1.   Digital computer plot of left ventricular diameter, aortic instantaneous and integral flow and left ventricular pressure averaging 10 consecutive beats in a conscious, resting animal.

Figure 2.   Digital computer plots of left ventricular pressure versus left ventricular internal diameter and integral aortic flow versus left ventricular diameter averaging the same 10 beats shown in Figure 1.

Figure 3.   Overlay of the 10 consecutive beats averaged in Figure 1 and 2.

Figure 4.   Solid lines are from top to bottom, left ventricular diameter, left ventricular pressure and aortic flow. Dots are the derivatives of the corresponding parameter. Data is from 10 averaged beats.

Figure 5.   Pressure versus diameter during the diastolic filling period in a conscious dog using average of 10 consecutive beats. The vertical broken lines divide the curve into three segments: to the left is the period of elastic recoil, in the center is the period of elastic reequilibration and to the right is the period of elastic opposition.

Figure 6.   Top shows diastolic pressure - diameter plot for control, isoproterenol and metaraminol states. Bottom shows same parameters at two different isoproterenol infusion rates.
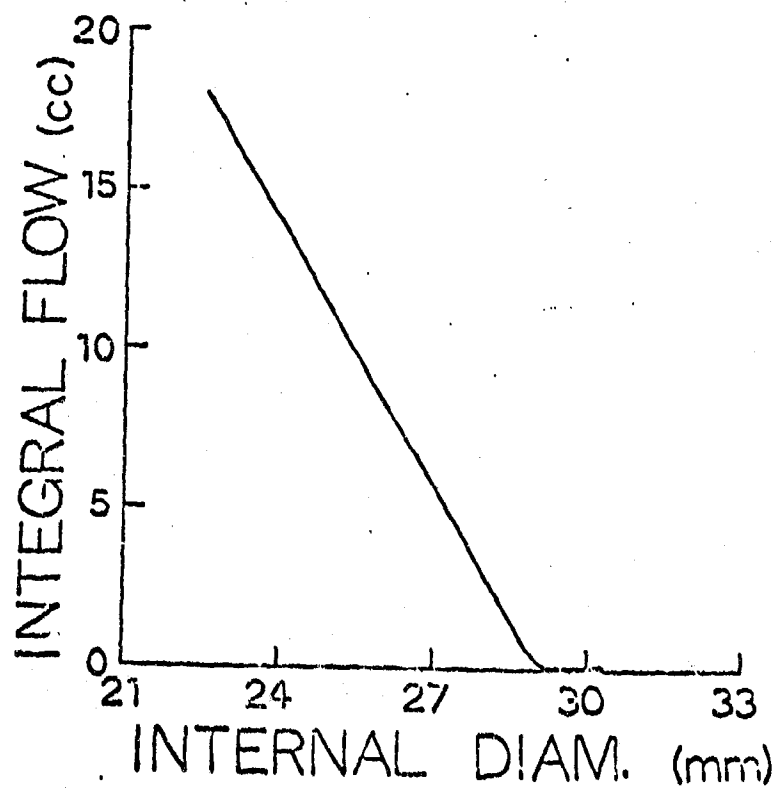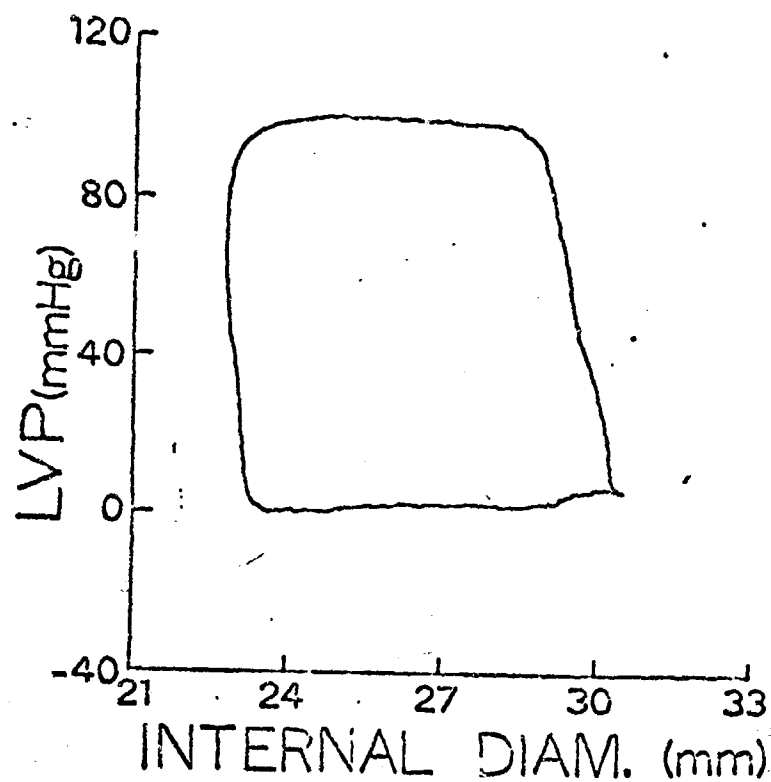
FIGURE 1.
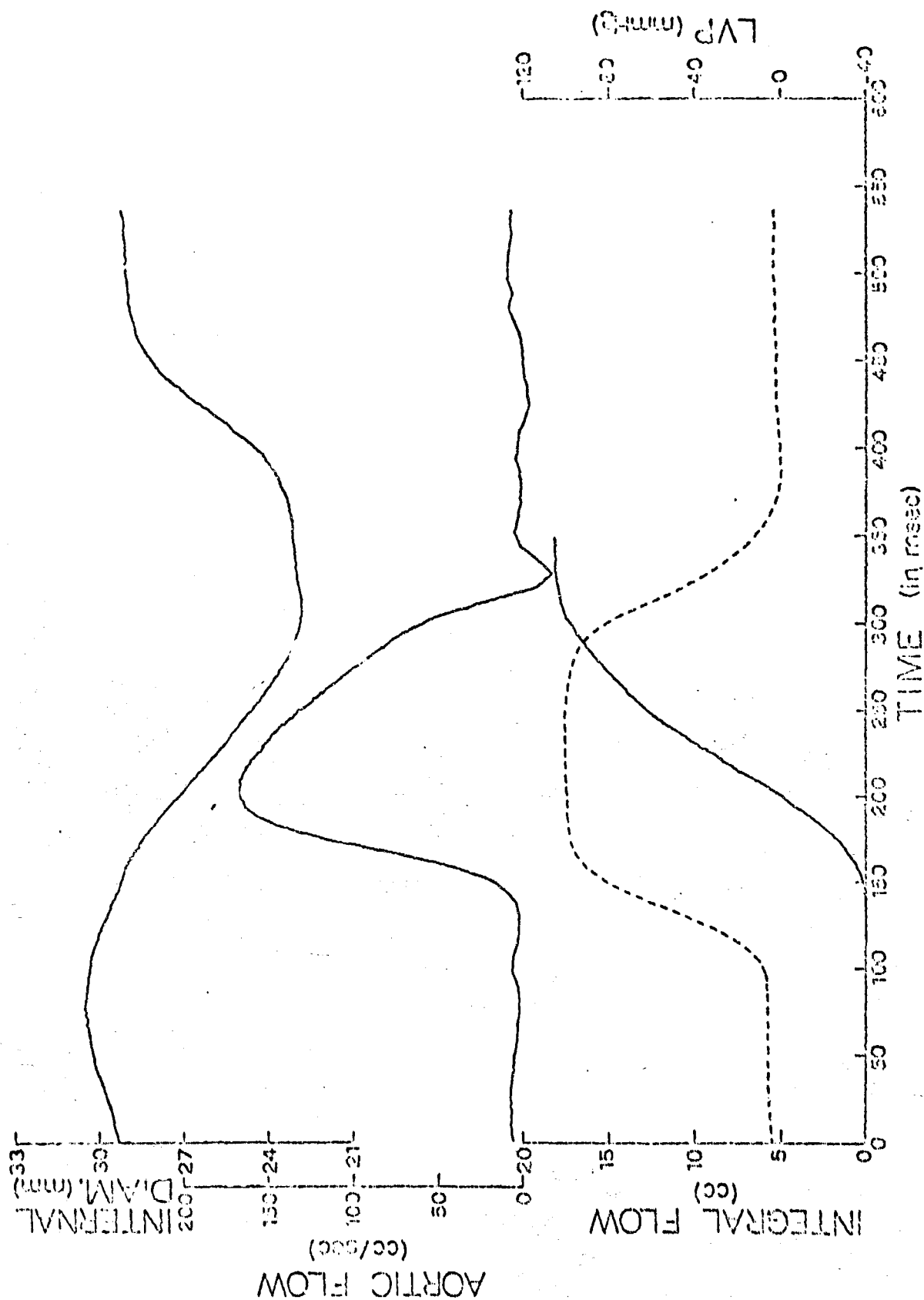
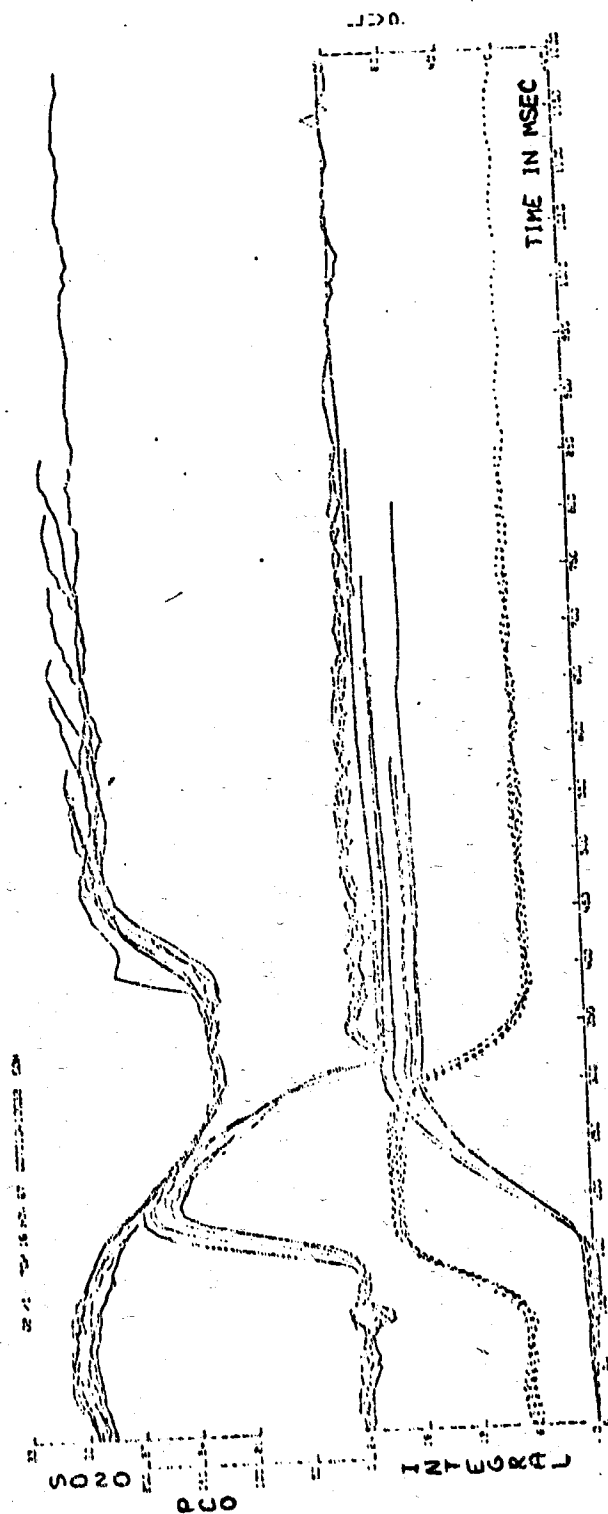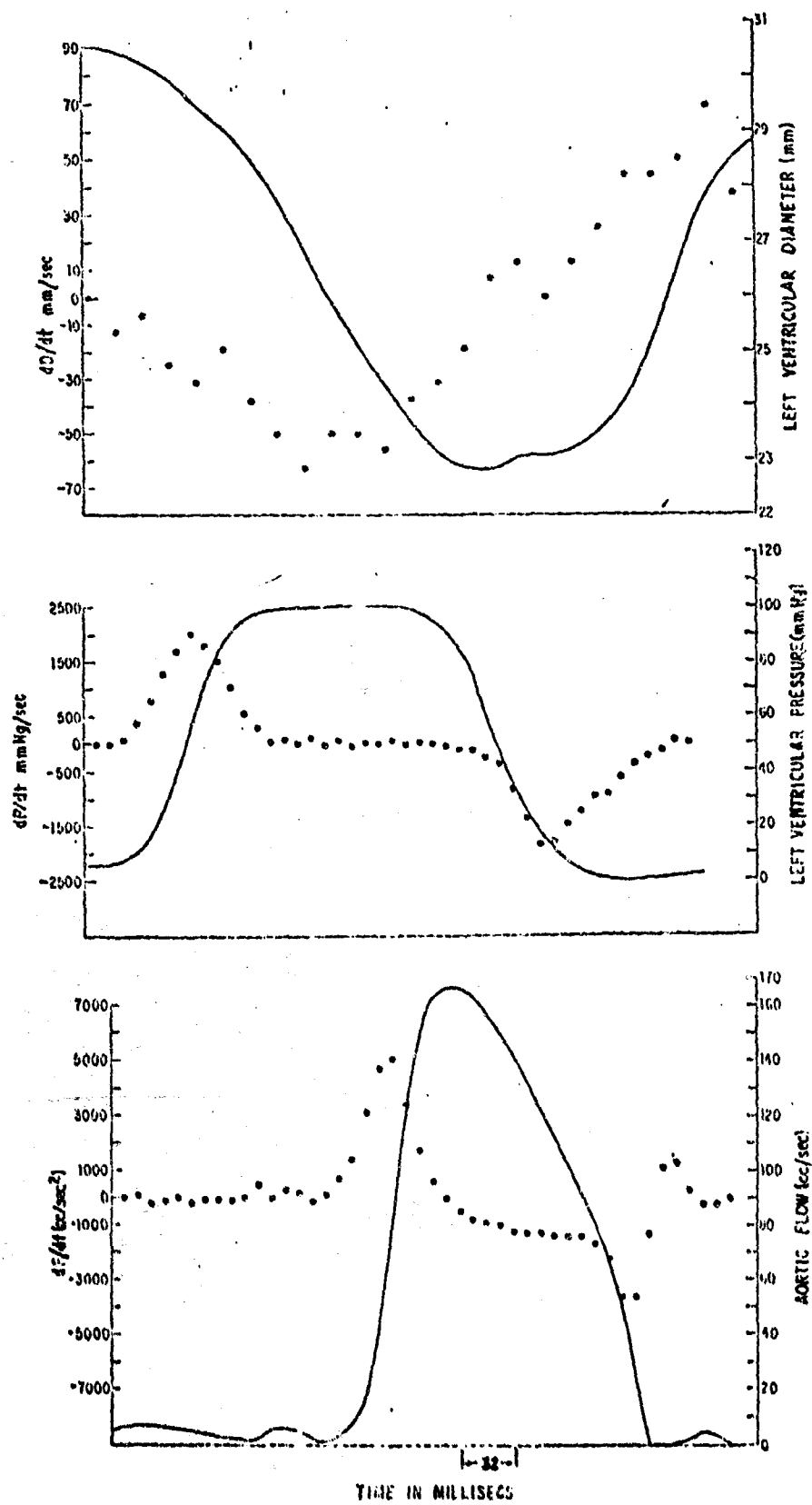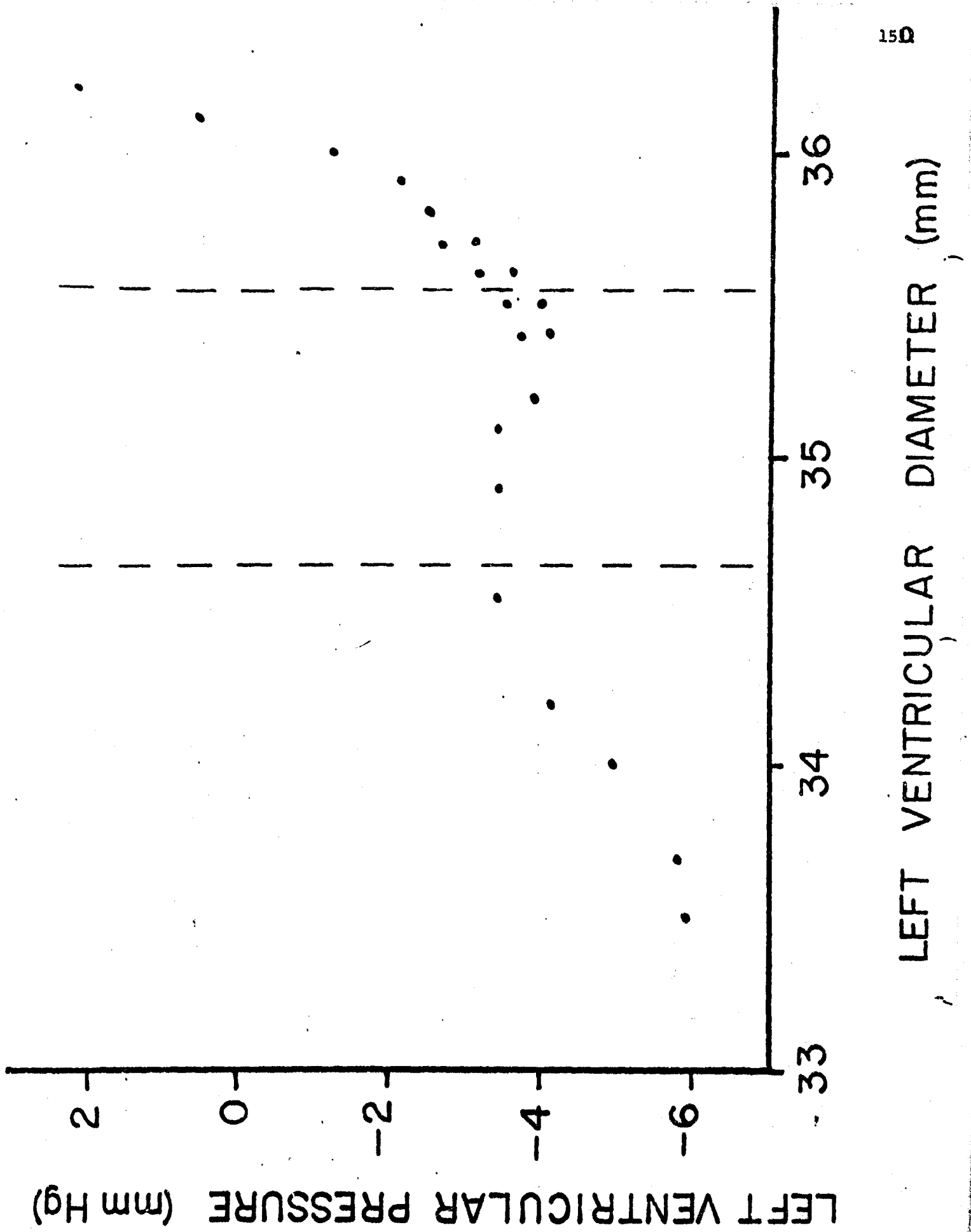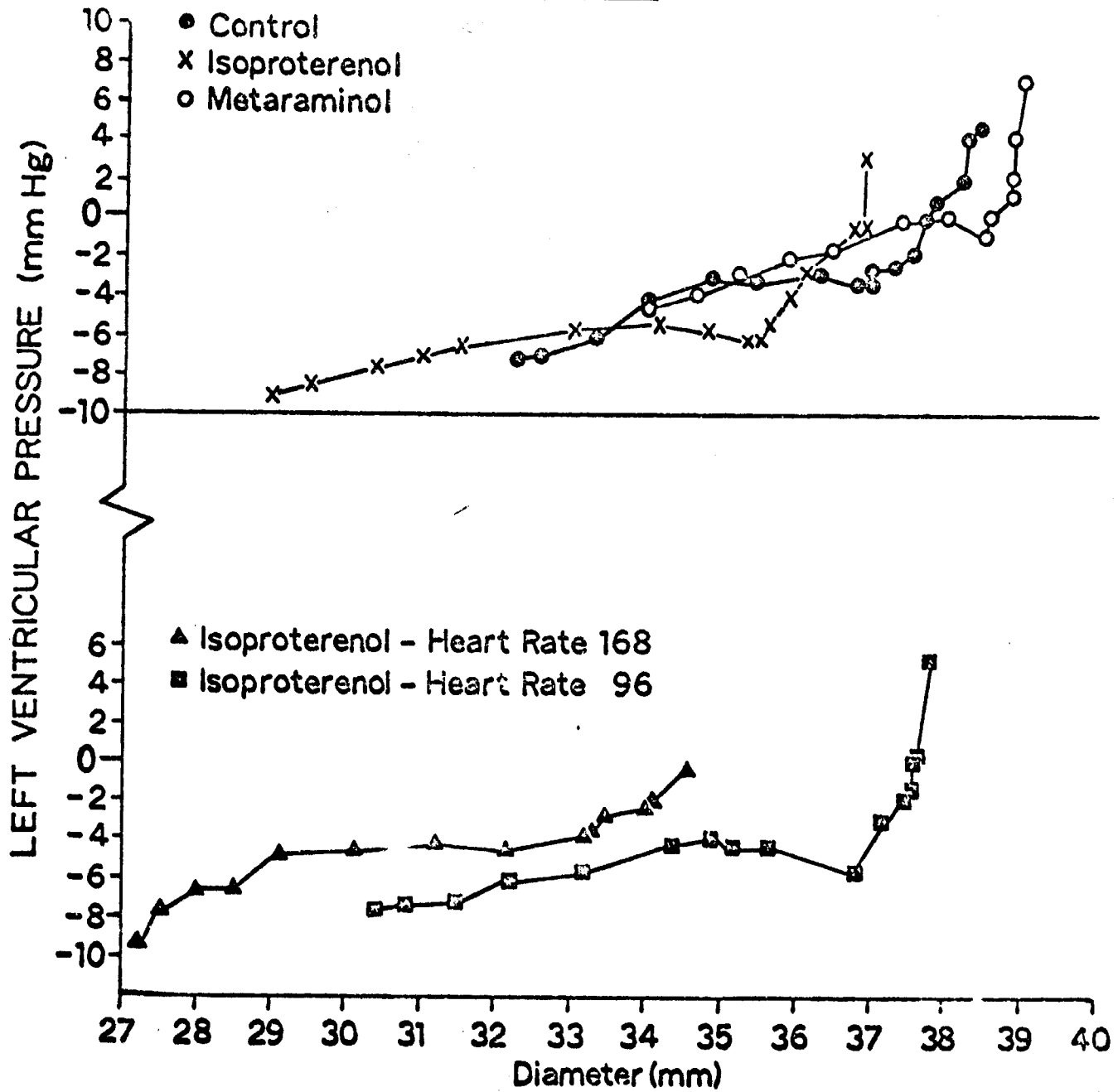01-70H   FIGURE 2.

FIGURE 3.

TIME IN MSEC

FIGURE 4.

Dog 425

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EFFECTS OF ALTERED AUTONOMIC CONTROL ON LEFT VENTRICULAR FUNCTION IN

CONSCIOUS DOGS

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Running Head: Autonomic Control of Left Ventricular Function

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ABSTRACT

BISHOP, VERNON S., AND LAWRENCE D. HORWITZ. Effects of altered
autonomic control on left ventricular function in conscious dogs.
Am. J. Physiol. In 8 conscious dogs, effects of beta-adrenergic,
vagal, and combined beta-adrenergic and vagal blockage on left ven-
tricular internal diameter, pressure, and outflow were measured
at rest and during acute volume loading. At rest, beta-adrenergic
blockage resulted in a decrease in heart rate with no change in
stroke volume but increased end diastolic and end systolic diameters,
whereas vagal blockage resulted in an elevated heart rate with re-
ductions in stroke volume, end diastolic and end systolic diameters.
Combined blockage, at rest, was associated with elevations in heart
rate, diminished stroke volume, and increases in end diastolic and
end systolic diameters. During acute volume loading, beta-adrenergic
blockage reduced peak heart rate and stroke volume, and elevated end
systolic diameter, whereas vagal blockage, despite an elevated
heart rate, did not alter peak stroke volume and reduced end dia-
stolic diameter. The response to acute volume loading in combined
blockage was characterized by reduction in peak stroke volume and
end diastolic diameter. Stroke volume was found to be dependent not
only on the initial fiber length but also on the sympathetic innerva-
tion. This was demonstrated by the increase in end systolic diameter
following beta-adrenergic blockage.

left ventricular function   vagal blockage

beta-adrenergic blockage   left ventricular diameter

The autonomic nervous system is an important factor in the regulation of cardiac performance. However, the manner in which autonomic activity affects the cardiac reserve and the hemodynamic mechanisms utilized in response to stress has not been extensively studied. Information has been particularly sparse concerning the influence of autonomic activity on dynamic left ventricular dimensions when the heart is under stress.

An estimation of cardiac reserve in conscious animals can be obtained by determination of ventricular output curves by rapid intravenous infusions of isotonic solution until a maximum cardiac output is reached (2, 11). By this means, cardiac performance can be reproducibly measured in a controlled laboratory setting by measuring stroke volume, heart rate and other pertinent parameters as a function of the increased filling pressure. Recently, an ultrasonic technique has been described for continuous measurement of left ventricular internal, transverse diameter in conscious dogs (7). The diameter measurement has proven to be an accurate index of left ventricular volume (1). To investigate the cardiac response to alterations autonomic innervation during the stress of acute volume loading, left ventricular diameter, pressure, stroke volume and heart rate were measured in conscious dogs under conditions of normal autonomic control, beta-adrenergic blockage, vagal blockage, and the combination of beta-adrenergic and vagal blockage, while ventricular output curves were performed.

METHODS

Instrumentation and Measurement of Variables

In 8 adult mongrel dogs, weighing 18-21 kg, sterile thoracotomies were performed under methoxyfluorane anesthesia. Two sonomicrometer transducers were implanted on the endocardial surface of the anterior and posterior left ventricular wall using the technique described by Horwitz, et al (7). This was a closed heart technique which required a single stab wound through the anterior wall. With correct implantation the transducers were in a plane perpendicular to the longitudinal (apex-to-base) axis of the left ventricle.

Through a second stab wound near the apex, a Whittaker #1017 solid state pressure transducer was implanted within the left ventricle. An electromagnetic flow probe was placed around the ascending aorta, an 18 gauge polyvinyl catheter was placed in the left atrial appendage, and a 9 gauge polyvinyl catheter was placed in the left jugular vein. The electrical leads and catheters were exteriorized at the back of the neck. All animals were allowed two weeks to recover from the effects of surgery before experiments were begun. When studied they could exercise normally, and no electrocardiographic abnormalities were present.

A sonomicrometer was used to continuously measure left ventricular internal diameter (7, 1). By shock exciting one of the piezoelectric crystal transducers, bursts of 5 mHz ultrasound were generated. The transient time for the ultrasound to traverse the distance between the two crystals was recorded. These readings were

convertible to distance, since the velocity of ultrasound in blood
(1.5 x 10^3 m/sec) is known.

A Medicon K2000 flowmeter was used to detect aortic flow. The
flow probes were calibrated in vitro before implantation and re-
checked after the animals were sacrificed. In all cases the two
calibrations agreed within 5%. The signal in late diastole was
assumed to represent zero flow.

A previously calibrated Whittaker #1017 solid state pressure
transducer was implanted near the apex of the left ventricle. The
calibrations were confirmed in vivo with a Dallons Telco catheter.
The calibrations did not change but the zero baseline drifted from
day to day. It was found that the mean left atrial pressure was
always within 1 mmHg of the measured left ventricular end diastolic
pressure; agreement within 0.2 mmHg has been reported by others (4).
Therefore, the zero baseline was corrected by adjusting the left
ventricular end diastolic pressure to equal the mean left atrial
pressure at rest.

Mean arterial pressures were recorded either by a percutaneous
puncture of the right femoral artery or through a catheter implanted
in the left internal mammary artery. Pressures were measured with
Statham P23Db strain gauges; with the animals lying on their right
sides, the midline of the sternum was the zero reference. Electro-
cardiograms were recorded with subcutaneous needle electrodes.

All signals were inscribed on a Type R Beckman Oscillographic
recorder (Fig. 1) and an Ampex FR1300 magnetic tape recorder. Tapes

were analyzed with a Philco 3000 digital computer after analogue-to-digital conversion. Left ventricular diameter, aortic flow, and the integral of the aortic flow were examined as a function of the R-R interval of the electrocardiogram. Integrated aortic flow and left ventricular pressure were computed as a function of left ventricular diameter. To improve the signal-to-noise ratio and to prevent bias in beat selection, ten consecutive beats were averaged.

The sonomicrometer and the flowmeter both were found to have phase lags of 40° at 20 Hz. The lag in the pressure record was assumed to be negligible, based on its electronic characteristics. Therefore, the three measurements could be compared in time since the lag in the dimension and flow measurements was less than the sampling interval of the computer (0.008 seconds).

Experimental Conditions

Control experiments. Resting measurements were obtained while the animal was lying quietly on its right side, unsedated and lightly restrained. Following these measurements, ventricular output curves were determined by rapidly infusing the animals with Tyrode's solution through the left jugular vein catheter (2, 11). A pressure bottle was used to adjust the rate of infusion so that a steady rise in left atrial and left ventricular end diastolic pressure occurred. Infusions were administered for 3-6 minutes and were maintained until the cardiac output reached a constant level, while the left atrial and left ventricular end diastolic pressure continued to rise. At least two days of rest were allowed after each control or experimental infusion.

Beta-adrenergic blockage. Propranolol (0.5 mg/kg - 1.0 mg/kg) was given intravenously. In unpublished tests, we found that these dosages were required to eliminate the chronotropic and inotropic response to isoproterenol in the conscious dog. Similar doses have been required in isolated preparations (3). Twenty minutes after administration of propranolol measurements were obtained, followed by the determination of a ventricular output curve. A control curve was usually obtained three days after beta-adrenergic blockage.

Vagal blockage. Acute, reversible, blockage was performed by freezing the right vago-sympathetic nerve after the left vago-sympathetic nerve had been cut (12). After sections 1 and 2 were completed, a stainless steel coil was placed around the right vago-sympathetic nerve under sodium pentobarbital anesthesia. The right vago-sympathetic nerve was temporarily blocked during studies by infusing refrigerated alcohol (approximately 15°C) through the coil.

Combination of beta-adrenergic and vagal blockage. This was performed after a series of control, beta block, and vagal block studies had been completed. Twenty minutes after an intravenous infusion of propranolol, the vagus was cold blocked. Immediate resting and ventricular output curve measurements were made.

RESULTS

Effects of Autonomic Intervention on Resting Hemodynamics

Beta-adrenergic blockage at rest. Eight animals were studied. As shown in Tables 1 and 2, blockage with propranolol resulted in significant increases in the end diastolic diameter (EDD), end systolic

when further increase in filling pressure (LVEDP) did not result in further increments in heart rate or stroke volume. Infusions performed without autonomic blockage resulted in approximately a doubling of heart rate, a 50% increase in stroke volume, an 8% increase in EDD, a 3% increase in ESD, and a 66% increase in left ventricular systolic pressure. The differences between the plateau values during control ventricular output curves and curves performed during autonomic blockage are shown in Table 3.

Effect of beta-adrenergic blockage on the left ventricular response to acute volume loading. Infusion of beta-blocked dogs, as in the normally innervated state, resulted in increases in heart rate, stroke volume, EDD, ESD and systolic pressure. However, comparison of the results at the plateau of the ventricular output curves with those in infusions without autonomic blockage demonstrated significant differences in the degree of the increment in several of these parameters. The mean increment in stroke volume was reduced by 15% (0.2 cc/kg-beat), the increment in heart rate was reduced by 14% (24 beats/min), and the increment in ESD was increased by 7% (2.0 mm) during beta-adrenergic blockade. The increments in EDD and systolic pressure were not significantly changed. The increment in cardiac output was decreased by 48% (86 cc/min-kg).

Effect of vagal blockage on the left ventricular response to acute volume loading. The resting heart rate after vagal blockage was extremely high and there was no further significant increase in heart rate during infusion; stroke volume, EDD, ESD and left ventricular

systolic pressure did increase in response to the volume load. Comparison of the variables at the plateau with infusions in which there was no autonomic blockage showed significant reductions in the increments in stroke volume and EDD but no difference in ESD. The heart rate, cardiac output and left ventricular systolic pressure were higher at the plateau of the vagal blocked ventricular output curves than in any of the other states.

Effects of beta-adrenergic blockage and vagal blockage on the left ventricular response to acute volume loading. The resting heart rate after combined blockage was relatively high but rose significantly during the infusion. Stroke volume, EDD, ESD and systolic pressure also increased. At the plateau the increments in stroke volume (-0.4 cc/min-kg) and EDD (-1.4 mm) were significantly reduced, however, whereas the increments in ESD and heart rate were not significantly changed from the results in the curves without autonomic blockage. The increment in cardiac output was significantly reduced (-69 cc/min-kg).

DISCUSSION

In the resting, conscious canine, beta-adrenergic blockage with propranolol consistently increased end diastolic and end systolic left ventricular diameter. These increases in diameter were not due to changes in afterload or heart rate. Systemic arterial pressure was usually not altered, and in some animals, particularly if the initial heart rate was slow, propranolol increased end diastolic and end systolic diameter without altering heart rate. Studies of

erect human subjects have previously demonstrated increased cardiac dimensions with beta-adrenergic blockage (5).

When control ventricular output curves, without autonomic blockage, were performed, the maximum stroke volume was attained through a substantial increment in end diastolic diameter and a considerably smaller increment in end systolic diameter. Since the maximum end diastolic diameter with propranolol was approximately the same as occurred during control ventricular output curves, the reduction in maximum stroke volume was due solely to an increased increment in the end systolic diameter. Sympathetic stimulation increases the maximum velocity of cardiac muscle fiber shortening and the extent of shortening in papillary muscle preparations (10). Therefore, it is likely that the increased stroke volume during acute volume loading in the presence of normal autonomic innervation is dependent upon sympathetic discharge, whereas beta-adrenergic blockage decreases the maximum stroke volume by limiting extent of fiber shortening, as reflected by the elevated end systolic left ventricular diameter. It has been suggested that propranolol depresses the heart independently of its blockage of beta-adrenergic receptors (8). However, a more recent study concluded that norepinephrine increases the rate of ionic calcium influx into the sarcoplasmic reticulum while increasing the maximum rate of shortening (9). Therefore, propranolol, in the dosages employed in this investigation, reduces contractility through blockage of beta-adrenergic receptors.

At rest, vagal blockage resulted in a large increase in heart
rate with simultaneous decreases in stroke volume, left ventricular
end diastolic pressure, and left ventricular end diastolic and end
systolic diameter. At the peak of the ventricular output curves,
vagal blockage did not reduce stroke volume despite the tachycardia
and a significant reduction in maximum end diastolic diameter. Al-
though volume loading increased left ventricular filling pressure,
the high heart rate with vagal blockage may have limited diastolic
filling. However, with sympathetic innervation unimpaired, vagal
blockage resulted in ejection of a normal stroke volume through
greater cardiac muscle fiber shortening, as indicated by the reduc-
tion in peak end systolic diameter in most animals.

When vagal and beta-adrenergic blockage were combined, resting
heart rate was high, but there was not a decrease in cardiac size
as occurred with vagal blockage alone. Instead, the tachycardia
was accompanied by an increase in end diastolic and end systolic
left ventricular diameter, a dimension change which resembled the
resting response to beta-adrenergic blockage alone. At the peak of
the ventricular output curves, the response resembled that of beta-
adrenergic blockage alone in that maximum stroke volume and heart
rate were usually reduced and end systolic diameter usually slightly
increased as compared with the control curves. Maximum end diastolic
diameter was significantly decreased, as occurred with vagal block-
age alone.

It is apparent that abolition of sympathetic innervation, whether

or not vagal activity is present, impairs the stroke volume response
to volume loading by decreasing the extent of cardiac muscle fiber
shortening. This is reflected by greater increments in end systolic
than end diastolic diameter during ventricular output curves performed
after administration of propranolol, with or without the addition of
freezing of the vago-sympathetic nerve. However, when sympathetic
innervation is intact the ability of the cardiac muscle fibers to
shorten substantially is preserved, even at high heart rates. Thus
maximum cardiac output was increased during vagal blockage because
the marked augmentation of heart rate did not prevent ejection of
the same maximum stroke volume as was attained during control ven-
tricular output curves. In vigorous exercise, when sympathetic
activity was presumably greater than in acute volume loading, we
have found comparable stroke volumes at much higher heart rates (6).
There appeared to be a tendency for end diastolic cardiac size to
be limited by high heart rates. The decreased end diastolic diameter
with vagal blockage can be explained on this basis. It is more diffi-
cult to understand the reduced end diastolic diameter during volume
loading with combined blockage, when the peak heart rate was less
than the control level, although in excess of that with beta-adren-
ergic blockage alone. It is possible that absence of beta-adrenergic
stimulation hampered diastolic filling at somewhat lower heart rates
than was the case when autonomic innervation was intact. The relatively
high resting heart rate in combined blockage may have contributed to the
lack of diastolic distention of the ventricle during infusion if there
was a slower rate of filling due to lack of beta-adrenergic stimulation.

Table 1

Mean Measurements of Left Ventricular Function

	CO(cc/min-Kg)	HR(b/min)	SV(cc/b)Kg	EDD(mm)	ESD(mm)	LVEDP(mmHg)	LVPSP (mmHg)
Control							
Rest	74±3	87±6	0.86±0.06	34.7±1.4	29.3±1.6	3.1±0.6	126±8
Plateau of ventricular output curves	200±14	160±10	1.26±0.08	37.6±1.3	30.3±1.6		147±9
Control							
Rest	76±6	80±3	0.97±0.08	33.4±1.6	28.9±1.4	3.3±0.6	120±7
Beta-Adrenergic Blockage							
Rest	65±5	73±5	0.91±0.08	35.6±1.4	30.5±1.4	5.7±0.6	125-
Plateau of ventricular output curves	136±13	132±10	1.07±0.09	37.2±1.1	31.2±1.0		138±7
Control							
Rest	96±11	110±4	0.88±0.11	30.6±1.7	27.1±2.0	2.7±0.4	124±10
Vagal Blockage							
Rest	102±9	192±5	0.54±0.04	28.4±1.9	24.6±1.8	1.1±0.4	131±11
Plateau of ventricular output curves	242±29	192±7	1.26±0.16	34.1±1.8	27.1±1.7		133±12
Control							
Rest	85±7	110±4	0.79±0.08	34.5±1.6	30.8±1.7	1.5±0.5	124±12
Beta and Vagal Blockage							
Rest	81±10	135±10	0.60±0.06	35.6±1.5	33.1±1.8	1.8±0.5	133±12
Plateau of ventricular output curves	135±11	142±7	0.94±0.05	38.2±1.5	33.2±1.7		.147±12

The average control hemodynamic parameters are shown at rest (Control) either before rapid infusion or before autonomic blockage; at rest after beta-adrenergic blockage, vagal blockage or the combination of beta-adrenergic and vagal blockage; and at the peak of the ventricular output curves (Plateau) after control resting, beta blockage, vagal blockage or the combination blockage. HR = heart rate, SV = stroke volume, EDD = end diastolic diameter, ESD = end systolic diameter, LVEDP = left ventricular end diastolic pressure, and LVPSP = left ventricular peak systolic pressure. ± = SEM

Table 2

Mean Changes Resulting From Autonomic Blockage at Rest

	CO cc/min-Kg	HR Beats/min	SV cc-beat-Kg	EDD mm	ESD mm	LVEDP mmHg	LVPSP mmHg
Beta-Adrenergic Blockage	-11±4*	-7±5	-0.07±0.02*	+0.9±0.2*	+1.6±0.4**	+2.2±0.4**	+6±4
n = 8							
Vagal Blockage	+6±9	+83±9**	-0.40±0.05*	-2.2±0.6*	-2.5±0.5*	-1.8±0.3**	+7±4
n = 6							
Beta-Adrenergic Blockage and Vagal Blockage	-4±8	+25±13	-0.19±0.04*	+1.1±0.3*	+2.3±0.8*	+0.3±0.4	+13±9
n = 5							

± = s.d.

* P<0.05

** P<0.01

Other symbols same as on previous tables.

Table 3

Changes From the Control at the Plateau of the Ventricular Output Curves

	CO cc/min-Kg	HR beats/min	SV cc/beat-Kg	EDD mm	ESD mm	LVPSP mmHg
Beta-Adrenergic Blockage	-59±12**	-24±7*	-0.20±0.04**	+0.5±0.3	+2.0±0.3**	-10±6
n = 8						
Vagal Blockage	+36±22	+43±7**	-0.12±0.11	-1.6±0.6*	-0.8±1.0	-11±
n = 6						
Beta-Adrenergic and Vagal Blockage	-69±17*	-17±14	-0.4±0.08**	-1.4±0.4*	+0.3±0.7	+9±5
n = 5						

Symbols same as on previous tables.

CONTROL PEAK INFUSION
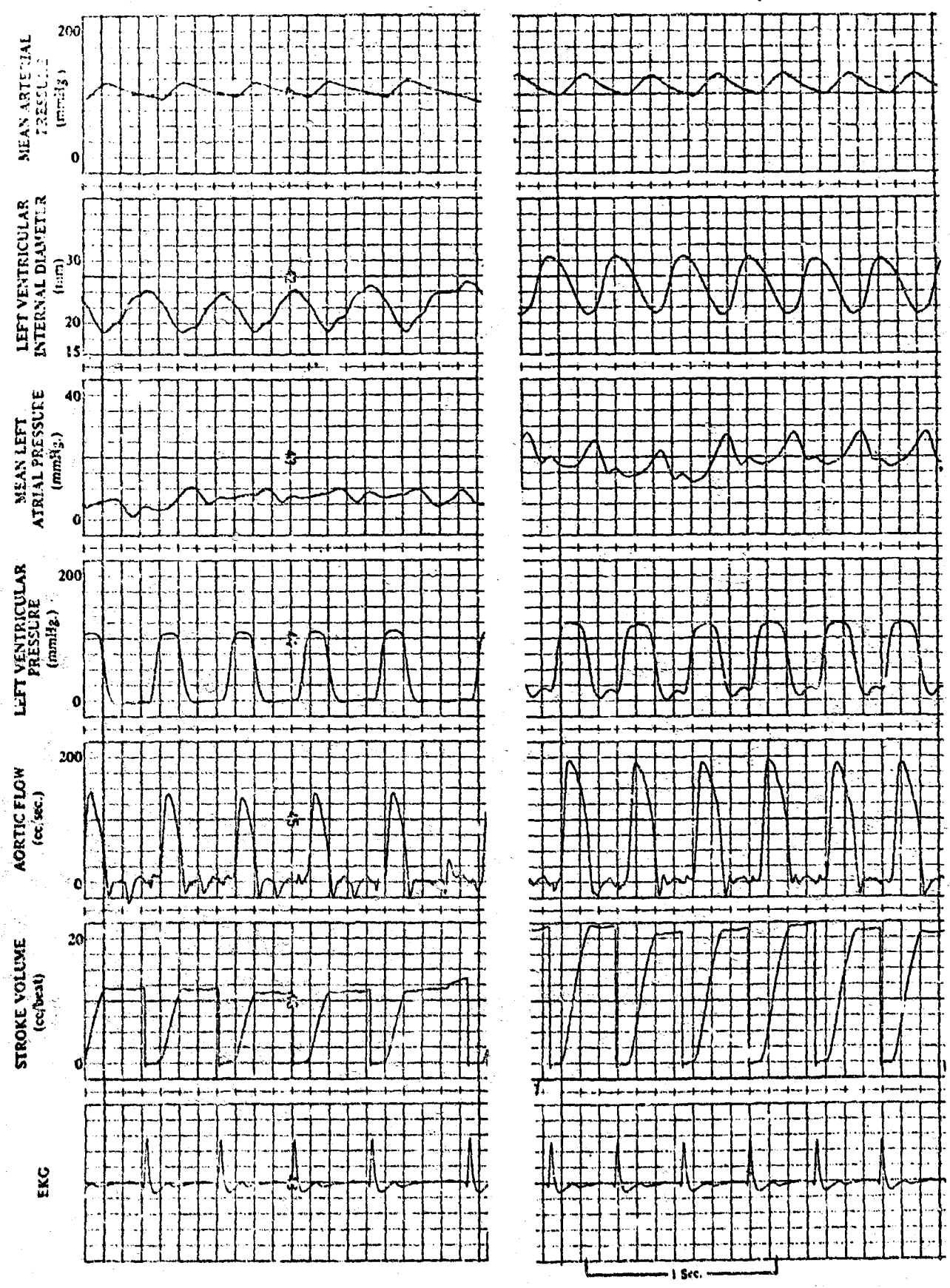
MEAN ARTERIAL PRESSURE (mmHg.)

LEFT VENTRICULAR INTERNAL DIAMETER (mm)

MEAN LEFT ATRIAL PRESSURE (mmHg.)

LEFT VENTRICULAR PRESSURE (mmHg.)

AORTIC FLOW (cc/sec.)

STROKE VOLUME (cc/beat)

EKG

1 Sec.

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HEMODYNAMIC EFFECTS OF NITROGLYCERIN AND AMYL NITRITE IN THE CONSCIOUS DOG*

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Running Title:

EFFECTS OF NITROGLYCERIN, AMYL NITRITE

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Abstract

The instantaneous hemodynamic effects of intravenous nitroglycerin (25 µg/kg) and amyl nitrite inhalation were compared in seven conscious mongrel dogs two weeks or more after implantation of electromagnetic flow probes around the ascending aorta and the insertion of polyvinyl catheters into the right atrium, left atrium and ascending aorta. The hemodynamic effects of the two drugs were identical. The decrease in mean arterial pressure, stroke volume, and mean atrial pressures and the increase in heart rate and cardiac output were all statistically significant.

In two conscious dogs continuous measurements of transverse internal left ventricular diameter were recorded by a sonomicrometer before and during drug administration. The end-diastolic diameter, the end-systolic diameter and the stroke excursion (end-diastolic minus end-systolic diameter) decreased from control values with both nitroglycerin and amyl nitrite. ($p < 0.05$).

In two dogs the heart rate was controlled by a right atrial bipolar pacemaker catheter and the experiment was repeated on four occasions with each drug. With the heart rate controlled at 210 beats/minute, both nitroglycerin and amyl nitrite produced an increment rather than a decrease in stroke volume, and an increase in stroke excursion, despite a decrease in end diastolic diameter.

Abstract (Cont'd.)

This study indicates that rapidly administered amyl nitrite and nitro-glycerin produce identical hemodynamic changes which result from vasodili-tation of resistance and capitance vessels and the baroreceptor reflex response to a sudden decrease in mean arterial pressure.

Introduction

Despite the widespread use of nitroglycerin and amyl nitrite as thera-
peutic agents in the treatment of angina pectoris and the frequent use of amyl
nitrite inhalation for evaluating cardiac murmurs, considerable controversy ex-
ists concerning the mechanism of action of these two pharmacologic agents
(Honig et al, 1960; Mason and Braunwald, 1965; Sharpey-Schafer and Gins-
berg, 1962; Kot et al, 1967; Bernstein, et al, 1966, and Perloff et al, 1963).
Much of the confusion concerning the effect of these drugs on the capacitance
and resistance vessels and on cardiac output is due to the fact that these drugs
are often compared in the same patient or in the same experimental animal when
given at different rates and by different route of administration.

In the present study, the hemodynamic effects of amyl nitrite inhalation
and intravenous nitroglycerin were compared for the first time in the conscious
dog during continuous monitoring of cardiac output, heart rate, mean arterial
pressure, atrial filling pressures and left ventricular internal diameter.

Methods

Seven mongrel dogs, 8 to 16 kilograms in weight, were selected for this
study. At the time of thoracotomy, an electromagnetic flow probe was placed
around the root of the ascending aorta and #18 polyvinyl catheters were positioned
in the left atrium through the left atrial appendage and in the right atrium through
the right jugular vein. A third polyvinyl catheter was positioned in the ascending
aorta through an internal thoracic artery. Flow probe leads and catheters were
exteriorized at the nape of the neck.

Methods (Cont'd.)

During the same operation, two of these animals had sonomicrometer transducers implanted on the left ventricular endocardium using the technique described by Horwitz and associates (1968). A needle carrying one transducer was thrust through a stab incision made in the anterior wall of the left ventricle. The wires of this transducer were pulled through the posterior wall until the transducer lay flat against the posterior endocardial surface. A second transducer was then pushed through the same incision and positioned against the anterior inner surface of the ventricle. With practice, transducers could be implanted in a plane perpendicular to the longitudinal axis of the left ventricle and across its greatest internal diameter.

All animals could exercise normally within two weeks after surgery and appeared to be in good health. None showed any myocardial damage on post mortem examination. Details on the instrumentation, its calibration and the care of these animals has been previously reported (Bishop et al, 1965; Horwitz et al, 1968; and O'Rourke, et al, 1969).

In all seven conscious animals, mean cardiac output was measured with an electromagnetic flowmeter (Biotronic, Model BL-610). Flow probes were calibrated in vitro using dialysis tubing before implantation and the calibration was checked when the animals were sacrificed. Late diastolic flow was assumed to be zero.

Mean arterial pressure was obtained by the polyvinyl catheter previously placed in the aorta or by percutaneous insertion of a needle into the femoral artery. Both arterial pressures and mean atrial pressures were measured with strain gauge

Methods (Cont'd.)

manometers (Statham P23 Db and P23 Bb); the sternal midline (with the dog lyong on his right side) was the zero reference. Electrocardiograms were recorded with subcutaneous needle electrodes over the sternum. The heart rate was continuously monitored by a cardiotachometer. All measurements were recorded on a polygraph (Offner Type R). Stroke volume was obtained by dividing the mean cardiac output by the instantaneous heart rate and was checked by planimetric integration of the aortic flow velocity curve.

After obtaining control records with the animal lying quietly on the laboratory table, amyl nitrite (5 minim ampules) was administered by inhalation or nitroglycerin (25 μg/kg) was given introvenously over a fifteen second interval. The administration of these drugs were alternated whenever possible. Before giving each drug, hemodynamic parameters were allowed to return to control values. The time interval between experiments was 30 to 60 minutes and the number of experiments performed on any given animal during one setting was dependant upon the stability of control hemodynamic parameters. If an animal became anxious following the administration of amyl nitrite and nitroglycerin, no further drugs were administered on that day. No tolerance to either amyl nitrite or nitroglycerin administration was observed in any animal.

In two of these seven unanesthetized animals continuous transverse internal diameter of the left ventricle was recorded by the sonomicrometer transducers which had been implanted on the endocardial surface. The transducers are two piezo-electric crystals; one crystal is shock excited at a high repetition rate (5000/sec) and the time required for each ultrasonic burst to pass from one crystal to the other is converted into a voltage suitable for recording. Resolution is high and drift is negligible.

Methods (Cont'd.)

Readings can be converted to distance because the velocity of ultrasound in blood is known (1.5×10^3 m/sec).

In the two dogs with implanted left ventricular sonomicrometer crystals, a bipolar pacemaker catheter was inserted into the right atrium through the polyvinyl catheter in the right jugular vein. The heart rate was kept constant (210 beats/min.), before and after the administration of amyl nitrite and nitroglycerin to each dog on two occasions.

Results

Hemodynamics

Table 1 summarizes the hemodynamic data obtained before and during the peak effect of amyl nitrite inhalation and intravenous nitroglycerin (35 experiments with each agent). The experimental data shown in Table 1 were obtained at the time of maximal decrease in mean arterial pressure following amyl nitrite or nitroglycerin administration in the experiments in which the heart rate was allowed to vary freely.

There was a significant increase in cardiac output and cardiac index (expressed in ml/min per kg. of body weight) with both amyl nitrite and nitroglycerin administration. In individual experiments the increase in cardiac output was greatest when there was a rapid and marked decrease in mean arterial pressure. This increase in cardiac output during the administration of both agents was due to a marked increase in heart rate and occurred despite a significant reduction in stroke volume.

Results (Cont'd.)

Hemodynamics (Cont'd.)

The maximal decrease in arterial pressure usually occurred between 30 and 45 seconds after the initiation of amyl nitrite inhalation, and 15 to 20 seconds following the intravenous administration of nitroglycerin. The mean arterial pressure in these unanesthetized animals returned to control values with both agents within five minutes. There was no overshoot. This is also true for the cardiac output, stroke volume and heart rate.

On several occasions the effect of amyl nitrite on arterial pressure was delayed because of the conscious animal's reluctance to inhale this agent. In these experiments there was an actual decrease in cardiac output and no significant increase in heart rate. This occurred despite a gradual reduction in mean arterial pressure of 15 to 20 mm Hg. It appears that in this situation there was little if any reflex tachycardia in response to the slow decrease in mean arterial pressure.

The reduction in stroke volume occurred not only when there was an increase in heart rate and cardiac output, but also in the experiments in which the effects of amyl nitrite were delayed and there was little change in heart rate.

Both mean left and right atrial pressures decreased significantly during the peak effect of amyl nitrite and nitroglycerin. This decrease in filling pressures occurred regardless of the heart rate response, but was more marked during tachycardia.

Ventricular Dimensions

Table 2 shows the results of intravenous nitroglycerin and inhaled amyl nitrite on continuously recorded er-diastolic and end-systolic internal left ventricular transverse diameters during five experiments in each of two animals when the heart rate was allowed to vary freely. Both agents caused a significant decrease in left ventricular end-diastolic diameter. The decrease in end-systolic diameter which occurred following intravenous nitroglycerin or inhalation of amyl nitrite, was also statistically significant. Since both agents produced a decrease in left ventricular end-diastolic diameter which was greater than the decrease in end-systolic diameter, the stroke excursion (end-diastolic minus end-systolic diameter) decreased significantly during the administration of each drug.

Pacing

Figure 1 shows two segments of a continuous record obtained from a conscious dog before and 20 seconds after the intravenous administration of nitroglycerin. During this experiment, the heart rate was controlled at 210 beats/minute by a bipolar pacemaker catheter in the right atrium. The decrease in mean arterial pressure of 20 mm Hg was associated with a rise in cardiac output of 460 ml/minute which was due to an increase in stroke volume of 2.2 ml/beat. Left atrial pressure declined slightly.

Figure 2 shows two segments of a similar record obtained from the same dog on the same day, before and 30 seconds after inhalation of amyl nitrite. Again, at a fixed heart rate of 210 beats/minute, the reduction in mean arterial pressure was accompanied by an increase in stroke volume of 2.6 ml/beat and a small decrease in left atrial pressure.

Figure 3 shows the effect of intravenous nitroglycerin on the transverse internal diameter of the left ventricle in a conscious dog whose heart rate was

Pacing (Cont'd.)

maintained at 210 beats/minute by a right atrial pacemaker. 20 seconds after intravenous nitroglycerin there was a decrease in both end-diastolic diameter (0.9 mm) and end-systolic diameter (1.6 mm). This reduction in heart size was accompanied by an increase in stroke excursion (EDD-ESD) of 0.7 mm. and an increment in stroke volume of 1.4 ml/beat. Identical changes occurred during the inhalation of amyl nitrite.

Discussion

Nitroglycerin

There has been considerable dispute concerning the effects of nitroglycerin on the cardiac output. Measurements made in patients receiving sublingual nitroglycerin have shown a rise (Starr et al, 1937; Wegeria et al, 1951), no change (Brachfeld et al, 1959), or a small decrease in cardiac output (Eldridge et al, 1955; Williams et al, 1965; Frick et al, 1968; Knobel et al, 1968). After sublingual nitroglycerin the decrease in arterial pressure is small and the reflex increase in heart rate minimal.

In the present study nitroglycerin was given intravenously in an attempt to produce a rapid decline in arterial pressure and a reflex increase in heart rate similar to that occurring after amyl nitrite inhalation. In contrast to sublingual nitroglycerin, intravenous administration produced a sig-

Discussion (Cont'd.)

Nitroglycerin (Cont'd.)

nificant increase in cardiac output. In patients in whom there is a rapid de-

cline in systemic arterial pressure after sublingual nitroglycerin a similar rise

in cardiac output occurs (Starr et al, 1937). These observations suggest that

when nitroglycerin produces a rapid and pronounced decrease in arterial pres-

sure, the resulting baroreceptor reflex action produces an increase in heart

rate, myocardial contractile force and cardiac output (Pinkerson et al, 1963).

Several studies have suggested that a reduction in ventricular size occurs

following the administration of nitroglycerin (Williams et al, 1965; Frick et al, 1968;

Brandt et al, 1952; Hoeschen et al, 1966). However, the present investigation

is the first to document a decrease in internal left ventricular dimensions follow-

ing nitroglycerin administration. When the heart rate was allowed to vary freely

there was a decrease in stroke excursion and stroke volume. In contrast, when

the heart rate was controlled by a right atrial pacemaker, the decrease in end-

systolic diameter and end-diastolic diameter was accompanied by an increase in

both stroke excursion and stroke volume. The increase in stroke excursion is

presumably the result of a decline in afterload, as well as the reflex release of

catecholamines.

These observations demonstrate that even in the absence of reflex tachy-

cardia and in the presence of a diminished end-diastolic diameter nitroglycerin

enhances left ventricular function.

We also noted definite decreases in mean right and left atrial pressures

resulting from the intravenous injection of nitroglycerin. This decrease in filling

Discussion (Cont'd.)

Nitroglycerin (Cont'd.)

pressures was less marked in the six experiments in which the heart rate was controlled by the pacemaker catheter. These results indicate that the decrease in stroke volume and left atrial pressure that occurs in experiments in which the heart rate is allowed to vary freely is due predominantly to the decline in left heart filling which accompanies tachycardia. The small decrease in mean left atrial pressure which occurred in the animals with the fixed heart rate is most likely due to improved left ventricular emptying mediated by the baroreceptor mechanism. Mason and Braunwald (1965) have previously shown that sublingual nitroglycerin causes venous dilitation, decreased venous return and a decreased stroke volume. However, in the intact animal, a significant decrease in arterial pressure is often accompanied by reflex venous constriction (Ross et al, 1961) as well as increased myocardial "contractility", suggesting that both direct venodilator effect and a reflex venoconstrictive effect may occur following the intravenous administration of nitroglycerin.

Amyl Nitrite

Previous studies have left unexplained the small and variable changes in stroke volume following amyl nitrite inhalation (Perloff et al, 1963; Hoeschen et al, 1966). In the present investigation there was a significant increase in cardiac output associated with the significant decrease in mean arterial pressure and reflex increase in heart rate. In all 35 experiments in which the heart rate was allowed to vary freely, the stroke volume fell during the maximal decrease in arterial pressure.

<center>Discussion (Cont'd.)</center>

Amyl Nitrite (Cont'd)

By contrast in the two paced animals the stroke volume and stroke excursion increased following amyl nitrite inhalation. These results indicate that the hemodynamic effects of amyl nitrite inhalation parallel those of intravenous nitroglycerin. When heart rate and consequently ventricular filling time are kept constant, amyl nitrite inhalation causes an increase in left ventricular emptying from a decreased end-diastolic diameter. When the heart rate is allowed to vary freely the reflex tachycardia limits ventricular filling and causes a reduction in stroke volume and stroke excursion despite an increase in contractile force (Pinkerson et al, 1963).

In four determinations the animal was reluctant to inhale amyl nitrite, and one to two minutes elapsed before the arterial pressure reached its lowest level. In these experiments there was no significant increase in heart rate and a decline in both stroke volume and cardiac output occurred. The decrease in stroke volume and stroke excursion as well as the associated decrease in left atrial pressure presumably was due to peripheral venodilitation. The significant decrease in both end-diastolic and end-systolic diameters during these four experiments further support this contention.

The effect of amyl nitrite on the capacitance vessels is still controversial. Using an acute occlusion technique Mason and Braunwald (1965) found an increase in venous tone in the human forearm following the inhalation of amyl nitrite. However, Sharpey-Schafer and Ginsberg (1962), using a similar method reported a decrease in venous tone in the human forearm both following amyl

Discussion (Cont'd.)

Amyl Nitrite (Cont'd.)

nitrite inhalation and during the intra-arterial injection of sodium nitrite.

These are probably two effects of nitrites on the venous tone: (1) a direct veno-

dilator effect and (2) a reflex venoconstrictor effect originating in the barore-

ceptors and mediated through efferent sympathetic nerves. This is supported by

the observation that either reserpine or guanethedine administration abolishes

the peripheral venoconstriction due to amyl nitrite inhalation (Mason and Braun-

wald, 1965).

Conclusion

We conclude from the present study that the pharmacologic agents nitro-

glycerin and amyl nitrite have an identical effect on cardiovascular hemodynamics

in the conscious animal.

When given rapidly these agents cause an early and marked reduction in

mean arterial pressure which results in a significant increase in heart rate, myo-

cardial contractile force and cardiac output. There is a decrease in atrial fill-

ing pressures due predominantly to the tachycardia but also to the increase in

myocardial contractility. Left ventricular dimensions diminish for the same rea-

sons. The effect on the venous circulation is most likely two fold: an initial

direct dilating effect which is immediately followed by venoconstriction medi-

ated by the baroreceptor mechanism.

On the other hand when the agents are given slowly, there is a delayed

and less marked decline in mean arterial pressure. This is accompanied by little,

if any increase in heart rate and a reduction in both stroke volume and cardiac

Conclusion (Cont'd.)

output. The decrease in stroke volume is due to a decline in venous return which results from venodilitation. This fall in venous return also causes a reduction in ventricular dimensions. —

The hemodynamic observations in the paced animals given both drugs demonstrate an increase in left ventricular function, independent of the Frank-Starling mechanism, which is due to both a decrease in left ventricular afterload and a reflex mediated increase in myocardial contractility.

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CONTROL   NITROGLYCERIN

Stroke Volume
(ml/beat)

14.8   17.0

Femoral Arterial   120
Pressure (mm Hg)   60
0

Left Atrial   20
Pressure   10
(mm Hg)   0

ECG

Heart Rate   240
(Beats/min.)   180
120
60
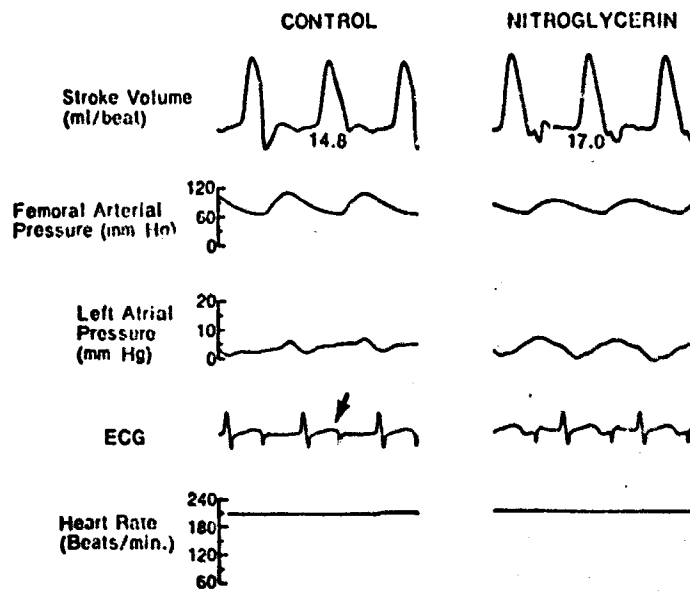
Fig. 1. Two segments of a continuous recording of hemodynamic parameters in a conscious dog before and 20 seconds after i.v. nitroglycerin. The heart rate is controlled by a right atrial pacemaker. Arrow shows pacemaker artefact. Stroke volume is obtained by integration of the aortic flow velocity curve (see text).

CONTROL   AMYL NITRITE

Stroke Volume
(ml/beat)

15.2   17.6

Femoral Arterial   120
Pressure (mm Hg)   60
0

Left Atrial   20
Pressure   10
(mm Hg)   0

ECG

Heart Rate   240
(Beats/min.)   180
120
60
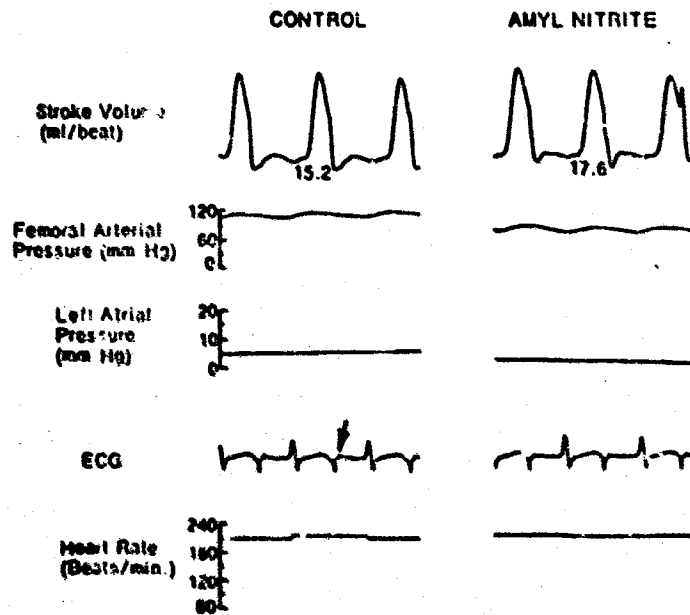
Fig. 2. Two segments of a similar recording before and 20 seconds after the inhalation of amyl nitrite.
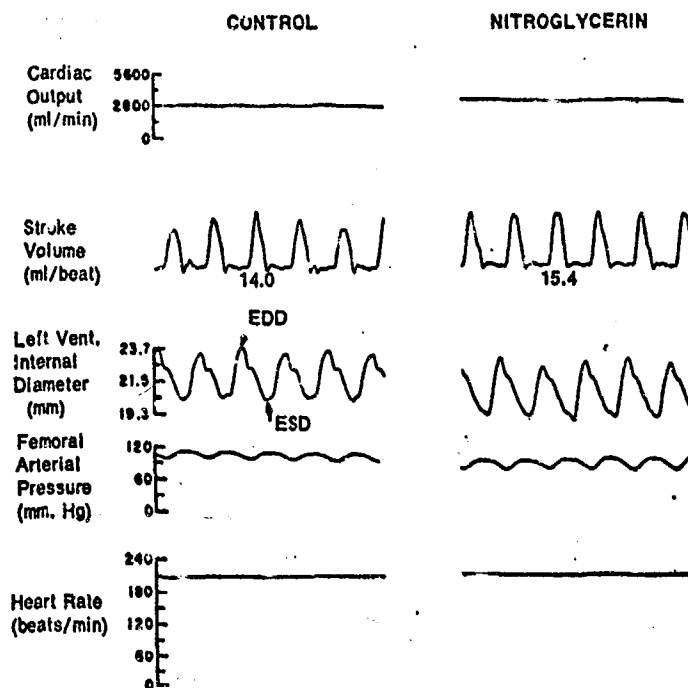
FIG. 3. Two segments of a continuous recording of hemodynamic parameters before and at the peak effect of i.v. nitroglycerin. EDD is end-diastolic diameter and ESD is end-systolic diameter (see text).

TABLE 1

Effect of amyl nitrite inhalation and intravenous nitroglycerin

on cardiovascular hemodynamics in the conscious dog

(35 determinations in seven dogs with each agent)

	Cardiac Output (ml/min)	Cardiac Index (ml/min/Kg)	Heart Rate (beats/min)	Stroke Volume (ml/beat)	Mean Arterial Pressure (mm Hg)	Left Atrial Pressure (mm Hg)	Right atrial Pressure (mm Hg)
Control	2208 ± 243.8^a	148 ± 13.2	123 ± 15.6	18.0 ± 2.9	108 ± 12.4	6.3 ± 2.1	4.2 ± 0.9
Nitroglycerin	2543 ± 249.3	171 ± 16.2	185 ± 27.1	13.8 ± 2.4	80 ± 18.2	3.7 ± 1.9	3.4 ± 0.7
p=	<0.001	<0.001	<0.001	<0.001	<0.001	<0.025	<0.025
Control	2140 ± 370.4^a	151 ± 19.7	125 ± 16.2	17.1 ± 2.1	100 ± 11.7	6.7 ± 2.1	3.8 ± 0.7
Amyl Nitrite	2412 ± 490.7	170 ± 34.3	183 ± 33.7	13.2 ± 1.7	80 ± 14.1	4.0 ± 2.0	2.3 ± 0.2
p=	<0.025	<0.001	<0.001	<0.001	<0.001	<0.025	<0.025

a - is one standard deviation.

TABLE 2

Effect of amyl nitrite inhalation and intravenous nitroglycerin

on ventricular dimensions

(5 determinations in each dog with each agent)

	End Diastolic Diameter (mm)	End Systolic Diameter (mm)	EDD – ESD (mm)
Control -- Dog I	28.8 ± 0.2^a	23.3 ± 0.2	5.5 ± 0.2
Nitroglycerin	25.5 ± 0.2	21.4 ± 0.1	4.1 ± 0.3
p=	< 0.001	< 0.001	< 0.001
Control -- Dog II	30.5 ± 0.3^a	26.1 ± 0.1	4.4 ± 0.3
Nitroglycerin	27.1 ± 0.2	23.7 ± 0.2	3.4 ± 0.2
p=	< 0.001	< 0.001	< 0.001
Control -- Dog I	28.9 ± 0.2^a	23.3 ± 0.2	5.6 ± 0.3
Amyl Nitrite	26.4 ± 0.2	22.7 ± 0.3	3.7 ± 0.2
p=	< 0.001	< 0.01	< 0.001
Control -- Dog II	30.6 ± 0.2^a	25.9 ± 0.2	4.7 ± 0.2
Amyl Nitrite	28.4 ± 0.1	25.4 ± 0.2	3.0 ± 0.1
p=	< 0.001	$p < 0.05$	$p < 0.001$

a - is one standard deviation

FOOTNOTES

The animals involved in this study were maintained in accordance
with the Guide for Laboratory Animal Facilities and Care" as published
by the National Academy of Sciences - National Research Council.

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VARIABLE EFFECT OF ANGIOTENSIN INFUSION
ON LEFT VENTRICULAR FUNCTION*

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ABSTRACT

Ventricular function curves derived from data obtained during increases in afterload with angiotensin were evaluated in six previously instrumented conscious dogs. Mean aortic flow, stroke volume, left ventricular pressure, heart rate and left ventricular transverse internal diameter were recorded before and during 12 continuous graded intravenous infusions of angiotensin. At peak angiotensin effect there were significant ($p < .001$) increases in L.V. systolic mean pressure (84 ± 8.1 to 120 ± 9.1 mm Hg) and L.V. end-diastolic pressure (4 ± 2.0 to 23 ± 2.8 mm Hg) and decreases in cardiac output ($1.88 \pm .40$ to $1.20 \pm .33$ liters per min.) and stroke volume (16.0 ± 3.4 to 11.0 ± 2.8 ml/beat). There were significant increments ($p < .01$) in end-diastolic diameter (1.7 mm) c ' end-systolic diameter (3.5 mm). Ventricular function curves were obtained by plotting stroke volume index or stroke work index on the ordinate and L.V. end-diastolic pressure on the abscissa. At least six data points were obtained from each experiment. Ventricular function curves were not comparable in the six dogs and on repeated determinations in individual dogs. There was no consistent relation between the end-diastolic pressure and the stroke volume index or stroke work index. These data suggest that changes in the ventricular function curve induced by increasing afterload with angiotensin are not a reliable index of ventricular function.

Additional Indexing Words

. erload

Function curves

Stroke work

Unanesthetized dogs

A method for analyzing the performance of the intact heart quantitatively and reproducibly has been a major goal of cardiovascular investigators for many years. Physiologic stresses that have been employed to assess ventricular function have included muscular exercise, alterations in preload, and progressive increases in afterload.[1-7] Ventricular function curves derived from data obtained during a progressive increase in arterial blood pressure by means of a graded intravenous infusion of angiotensin have been used in experimental animals and man to assess ventricular performance in the intact heart.[5-9] In these studies, curves relating stroke volume index or stroke work index to end diastolic pressure are usually constructed from two, three, and sometimes four data points. The presence of normal or diminished left ventricular function is often predicated upon the resulting ventricular function curve. The purpose of the present study was to determine if ventricular function curves obtained during increases in afterload with angiotensin provide a reliable index of left ventricular performance, and to evaluate the effect of intravenous angiotensin on continuously recorded ventricular dimensions.

Methods:

Six mongrel dogs, 10 to 18 kg in weight, were selected for this study. At the time of thoracotomy, an electromagnetic flow probe was placed around the root of the ascending aorta and 18 gauge polyvinyl catheters were positioned in the left atrium through the left atrial appendage and in the superior vena cava through the right jugular vein. Flow probe leads and catheters were exteriorized at the back of each animal's neck.

During the same operation, sonomicrometer transducers were implanted on the left ventricular endocardium using the technique described by Horwitz and associates.[10] A needle carrying one transducer was thrust through a stab incision in the anterior wall of the left ventricle. The wires of this transducer were pulled through the posterior wall until the

Methods (Cont'd.)

transducer lay flat against the posterior endocardial surface. The second transducer was then pushed through the same incision and positioned against the anterior surface of the ventricle. The transducers were implanted in a plane perpendicular to the longitudinal axis of the left ventricle and across its greater internal diameter. In addition, a solid state pressure transducer (Microsystems 1017) was implanted in the left ventricular apex. The animals were allowed to recover at least three weeks after surgery and could exercise normally at the time of experimentation. None showed any myocardial damage on post mortem examination.

In all six conscious animals mean cardiac output was measured with an electromagnetic flow meter (Medicon K-2000). Flow probes were calibrated before implantation with dialysis tubing and after implantation the cardiac output agreed within 10% to that simultaneously obtained by means of an indocyanine dilution curve. Mean diastolic flow was assumed to be zero.

The left ventricular pressure transducer was calibrated before implantation in a pressure jar against a column of mercury and after implantation with the pressure obtained with a #8 NIH catheter passed from the femoral artery into the left ventricle. The left atrial pressure was measured with a Statham P 23 Bb stain gauge (the sternal midline with the dog lying on his right side was the zero reference). Electrocardiograms were recorded from subcutaneous electrodes over the sternum. The heart rate was continuously monitored by a cardiotachometer. All measurements were recorded on a polygraph (Offner type R). Stroke volume was obtained by dividing the mean cardiac output by the instantaneous heart rate and was checked by planemetric integration of the aortic flow velocity curve. Left ventricular systolic mean pressure was derived from planimetry of the left ventricular pressure curve, and the left ventricular transverse internal diameter was recorded by means of

Methods (Cont'd.)

a portable sonomicrometer. Left ventricular stroke work was calculated from the form:

$$SW = \frac{SV \times (LVS - LVEDP) \times 1.36}{100}$$ where SV = stroke volume in ml; LVS = mean left ventricular pressure during ejection in mm Hg and LVEDP = left ventricular end diastolic pressure in mm Hg.

After obtaining control records with the animal lying quietly on the laboratory table, intravenous infusion of angiotensin was begun at a rate of one microgram per minute. Hemodynamic parameters were monitored continuously and the angiotensin infusion rate was increased at two to three minute intervals until arterial pressure increased and ventricular end diastolic pressure rose between five and ten mm Hg. The infusion rate was subsequently further increased until left ventricular end diastolic pressure increased by another five to ten mm Hg. The rate of angiotensin infusion in individual experiments varied between one and ten micrograms per minute. The duration of the angiotensin infusions averaged twenty minutes. Twelve experiments were performed in the six conscious unrestrained animals (two determinations in four dogs, three in one dog and one experiment in the sixth dog).

Left ventricular function curves were constructed from the data obtained during angiotensin infusion by plotting stroke volume index or stroke work index on the ordinate and left ventricular end diastolic pressure on the abscissa. Stroke volume and stroke work were expressed both per kilogram of body weight and per square meter of body surface are in order to compensate for variation in animal size. Body surface area in square meters was obtained by multiplying 2/3 of the animal's weight by 0.112[11].

RESULTS

Hemodynamics

Four segments of a continuous recording of cardiac output, left ventricular pressure,

Hemodynamics (Cont'd.)

internal diameter and heart rate during a 15 minute graded intravenous infusion of angiotensin are shown in Figure 1. A progressive decrease in mean cardiac output and stroke volume accompanied the progressive increase in left ventricular end diastolic pressure and left ventricular mean systolic pressure during the infusion of angiotensin. Left ventricular transverse end diastolic diameter and end systolic diameter increased and the stroke excursion (end diastolic minus the end systolic diameter) decreased. In this experiment, the heart rate decreased from 117 to 105 beats/minute. Presumably, this change in heart rate is mediated by the baroreceptors in response to the increase in left ventricular afterload.

The peak effect of intravenous angiotensin infusion on cardiac output, stroke volume, and left ventricular pressures in these twelve experiments is summarized in Table 1. Left ventricular mean systolic pressure increased from 84 to 120 mm Hg and mean left ventricular end diastolic pressure from 4 to 23 mm Hg. ($p < .00$); At the peak of the hypertensive effect cardiac output fell 680 ml per minute and the cardiac index 53 ml per kg per minute ($p < .001$). The average stroke volume decreased from 16.9 to 11.0 ml. Although the heart rate tended to decrease with each increment in the rate of angiotensin infusion, there is no statistically significant difference between the mean control heart rate (112 $\pm$ 10.4 beats/minute) and the heart rate at peak angiotensin effect (109 $\pm$ 13.1 beats/minute).

Figure 2 shows the peak effect of intravenous angiotensin on left ventricular transverse internal diameter. In all 12 experiments end diastolic diameter increased (1.7 $\pm$ 0.5 mm, $p < .01$) as did the end systolic diameter, (3.5 $\pm$ 0.8 mm., $p < .01$). Therefore, stroke excursion (end diastolic diameter minus end systolic diameter) decreased significantly during angiotensin infusion (1.8 $\pm$ 0.4 mm, $p < .01$). In all twelve experiments there is an excellent correlation between stroke excursion and stroke volume ($r = +0.97$). The transverse internal

RESULTS (Cont'd.)

Hemodynamics (Cont'd.)

diameter reflects the volume of the left ventricle since the stroke volume is linearly related to the left ventricular internal diameter during systole[12].

Function Curves

Three ventricular function curves obtained from three different conscious animals during similar rates of angiotensin infusion, are shown in Figure 3. Left ventricular stroke work index is plotted against left ventricular end diastolic pressure. The hemodynamic response to the increase in left ventricular afterload produced by intravenous angiotensin was different in each animal. This variability in ventricular function curves persists when either stroke volume index or minute work index is substituted on the ordinate for stroke work index.

Three different ventricular function curves obtained from the same conscious animal on three different days are depicted in Figure 4. Stroke work is plotted against the left ventricular end diastolic pressure. The three function curves were obtained from experiments performed 24 hours apart. Although control values for stroke work and left ventricular end diastolic pressure are similar, the hemodynamic response to angiotensin infusion was variable in the same conscious animal on three different occasions. The ventricular function curve derived from data obtained on the second day is strikingly depressed as compared to the function curves obtained on the day before and the day after this experiment. This substitution of stroke volume or minute work for stroke work as the ordinate does not improve the variability of the ventricular function curve in these three experiments.

The variation in the ventricular function curves obtained from the whole group of

RESULTS (Cont'd.)

Function Curves (Cont'd.)

animals is shown in Figure 5. These data were obtained from all 12 experiments. At any given left ventricular end diastolic pressure there is considerable scatter in the stroke work index. The spread is even greater for stroke work in absolute terms or for stroke work per kg of body weight. A similar variability is seen when stroke volume index is plotted against left ventricular end diastolic pressure (Figure 6).

In individual experiments the initial stroke volume response to intravenous angiotensin infusion varied. In five experiments there was an initial increase in stroke volume and in seven an initial decrease. In all twelve experiments the stroke volume was less than the control value at left ventricular end diastolic pressures of 18 mm Hg or greater.

DISCUSSION

Ventricular function curves have been used to quantitate cardiac performance during rapid intravenous fluid infusions in anesthetized and conscious animals by measuring cardiac output, stroke volume or stroke work as a function of ventricular filling pressure[1,2,3,12]. Ventricular output curves derived from data obtained during a rapid increase in preload are reproducible in the same conscious animal from day to day and the plateau of the function curve varies by less than 3.5% in a group of unanesthetized dogs[2,12]. Because of the potential hazard of the rapid administration of a large fluid load to patients with cardiac disease, increasing afterload has been proposed by many investigators as a means of obtaining multiple ventricular function curves[4,5,7-9]. Intravenous angiotensin infusion has been the method most frequently used in the experimental animal and in man to increase left ventricular afterload while recording changes in left ventricle mean systolic and end diastolic pressures as well as heart rate and cardiac output.

DISCUSSION (Cont'd.)

The cardiac output during intravenous angiotensin is usually obtained by the indicator dilution technique or by use of the Fick principle. Therefore, only 2, 3, and sometimes 4 data points are obtained for plotting stroke volume index or stroke work index against increasing ventricular end diastolic pressure. In the present study, designed to evaluate the reliability and reproducibility of ventricular function curves obtained during angiotensin infusion, cardiac output was recorded continuously by a previously placed electromagnetic flow probe. At least six data points were obtained in each experiment. The results indicate that ventricular function curves obtained during an increase in afterload with intravenous angiotensin may be considerably different on several occasions in the same animal and are quite variable in a similar group of animals. This variability is due primarily to inconsistent changes in stroke volume during the elevation in left ventricular filling pressure with angiotensin. These data suggest that ventricular function curves obtained by increasing afterload with angiotensin may not be a reliable index of left ventricular performance.

The present study is the first to report the effect of angiotensin infusion on continuously recorded left ventricular internal dimensions in the unanesthetized animal. The progressive increase in left ventricular systolic mean pressure is associated with an increase in left ventricular end diastolic diameter and end systolic diameter. The stroke excursion decreases as does the simultaneously measured stroke volume. Thus increasing afterload with angiotensin produces a decrease in the left ventricular ejection fraction (SV/EDD) and an increase in the residual fraction (ESD/EDD). There is a much greater increase in left ventricular end-diastolic pressure for each increase in end-diastolic diameter with angiotensin than is seen during continuous monitoring of these parameters when preload is increased by the rapid intravenous infusion of 200-400 ml of Tyrode's solution over a two minute interval[12]. This suggests an acute decrease in left ventricular compliance ($\Delta EDD/\Delta EDP$)

DISCUSSION (Cont'd.)

during the increase in afterload with angiotensin.

The effects of intravenous angiotensin on left ventricular hemodynamics reported in this study are similar to those reported by others. Most investigators[13-17] have recorded a decrease in cardiac output and stroke volume accompanying the increase in systemic vascular resistance and left ventricular diastolic pressure. These results have been found in unanesthetized research animals[13], normal human subjects[14-16] and patients with heart disease.

However, other observers[5,7,8,18] have reported an increase or no change in stroke volume during angiotensin infusion. In our experiments, there was often a small transient increase in stroke volume early in the course of angiotensin infusion which was followed by a progressive decline in the volume of blood ejected per beat. This momentary increase in stroke volume frequently accompanied a transient decrease in heart rate, the cardiac output remaining unchanged or decreasing slightly.

The decrease in stroke volume observed in this study may be due to: (1) Impeded left ventricular ejection due to the increase in afterload; (2) Vagal depression of the myocardium mediated by the baroreceptors in response to arterial hypertension; (3) angiotensin induced coronary vasoconstriction; (4) a direct negative inotropic effect produced by angiotensin.

> (1) The performance of the intact left ventricle as reflected in the
> stroke volume is profoundly influenced by the afterload alone.
> With other variables held constant, such as heart rate and fill-
> ing pressures, a progressive increase in aortic pressures produces
> a decline in stroke volume and the peak velocity of ejection[19].

DISCUSSION (Cont'd.)

(1) The normal left ventricular response to progressive proximal aortic obstruction consists of an increase in left ventricular systolic pressure, end-diastolic pressure and end-diastolic volume associated with a fall in cardiac output[4].

(2) In 1960, Segel, Harris and Bishop[15] showed that pretreatment with atropine prevented or diminished the decline in cardiac output following intravenous angiotensin in four subjects with normal cardiovascular and respiratory systems. In 1967, Nolan, Cobb and Thompson[14] obtained similar results in three patients without heart disease. Both studies suggest that stimulation of the arterial baroreceptor reflexes by increasing afterload is at least partially responsible for the reduction of cardiac output associated with angiotensin infusion.

(3) Many investigations in research animals and recent studies in patients with heart disease have indicated that angiotensin constricts the coronary vessels, increases the myocardial oxygen consumption and decreases the coronary venous oxygen content[17,20-22].

(4) The occurrence of direct inotropic effect with angiotensin are disputed. In the isolated cat pappilary muscle a consistent, concentration-dependent, direct positive action on the strength of ventricular contraction has been demonstrated with angiotensin.[23] However, others have shown little

DISCUSSION (Cont'd.)

(4) direct effect of angiotensin on myocardial contractility and

indirect depression of contractility secondary to coronary

constriction[21].

We conclude from the present study that a continuous graded intravenous infusion

with angiotensin produces an increase in left ventricular mean systolic and end-diastolic

pressures, an increment in end-diastolic diameter and a greater increase in end-systolic

diameter. This is associated with a decrease in cardiac output, stroke volume and stroke

excursion and no significant change in the heart rate. In individual experiments there is

a transient increase in stroke volume prior to its decline. Ventricular function curves ob-

tained from this data vary significantly when compared in similar animals of the same spe-

cies and may differ considerably in the same conscious animal on different determinations.

These data suggest that ventricular function curves obtained by this method provide an

unreliable index of ventricular function. Whether this is true only with angiotensin in-

fusion or also occurs during increases in afterload with other interventions such as intra-

venous methoxamine or phenylephrine remains to be answered.

Footnote:

The animals involved in this study were maintained in accordance with the "Guide for Laboratory Animal Facilities and Care" as published by the National Academy of Sciences – National Research Council.

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TABLE 1

AVERAGE LEFT VENTRICULAR HEMODYNAMICS IN THE 12 EXPERIMENTS

DURING THE CONTROL STATE AND

AT THE PEAK HYPERTENSIVE EFFECT OF ANGIOTENSIN

	CONTROL	PEAK ANGIOTENSIN	p VALUE
Left Ventricular Systolic Mean Pressure (mm Hg)	84 + 8.2*	120 + 9.2	< .001
Left Ventricular End-Diastolic Pressure (mm Hg)	4 + 2.0	23 + 2.7	< .001
Cardiac Output (ml/min)	1880 + 398	1200 + 334	< .001
Cardiac Index (ml/min/kg)	146.8 + 8.6	93.8 + 26.9	< .001
Stroke Volume (ml/beat)	16.9 + 3.4	11.0 + 2.7	< .001
Heart Rate (beats/min)	112 + 10.4	109 + 13.1	> .5

*is one standard deviation

LEGENDS

Figure 1:

Four segments of a continuous recording obtained during a 15 minute infusion with angiotensin. Stroke volume is obtained by planimetry of the aortic flow velocity curve.

Figure 2:

Average changes (Δ) in left ventricular transverse internal diameter at the peak hypertensive effect of intravenous angiotensin. EDD = end-diastolic diameter; FSD = end-systolic diameter; EDD - ESD - stroke excursion; SEM = standard error of the mean.

Figure 3:

Three ventricular function curves in three different conscious dogs.

Figure 4:

Three ventricular function curves in the same conscious dog on three different days

Figure 5:

Relation of stroke work index to ventricular end-diastolic pressure in the twelve experiments. 1 S. D. = 1 standard deviation. N = number of observations.

Figure 6:

Relation of stroke volume index to ventricular end-diastolic pressure in the twelve experiments. 1 S. D. = 1 standard deviation. N = number of observations.

	CONTROL	5 MINUTES	10 MINUTES	15 MINUTES
CARDIAC OUTPUT (L/min)	2.65	1.92	1.95	1.35
STROKE VOLUME (ml/beat)	22.7	20.4	17.5	12.8
L. VENT END-DIASTOLIC PRESSURE (mm Hg)	2	8	16	22
L. VENT MEAN SYSTOLIC PRESSURE (mm Hg)	82	109	118	124
L. VENT INTERNAL DIAMETER (mm)	26.0 / 21.1	28.4 / 24.1	29.0 / 25.0	28.6 / 25.5
HEART RATE (beats/min)	117	94	106	105

|←—ANGIOTENSION   INFUSION ——————————→|

FIG.
217

STROKE WORK – (gm–M)

Day 1
Day 2
Day 3

LEFT VENTRICULAR END-DIASTOLIC PRESSURE
(mmHg)
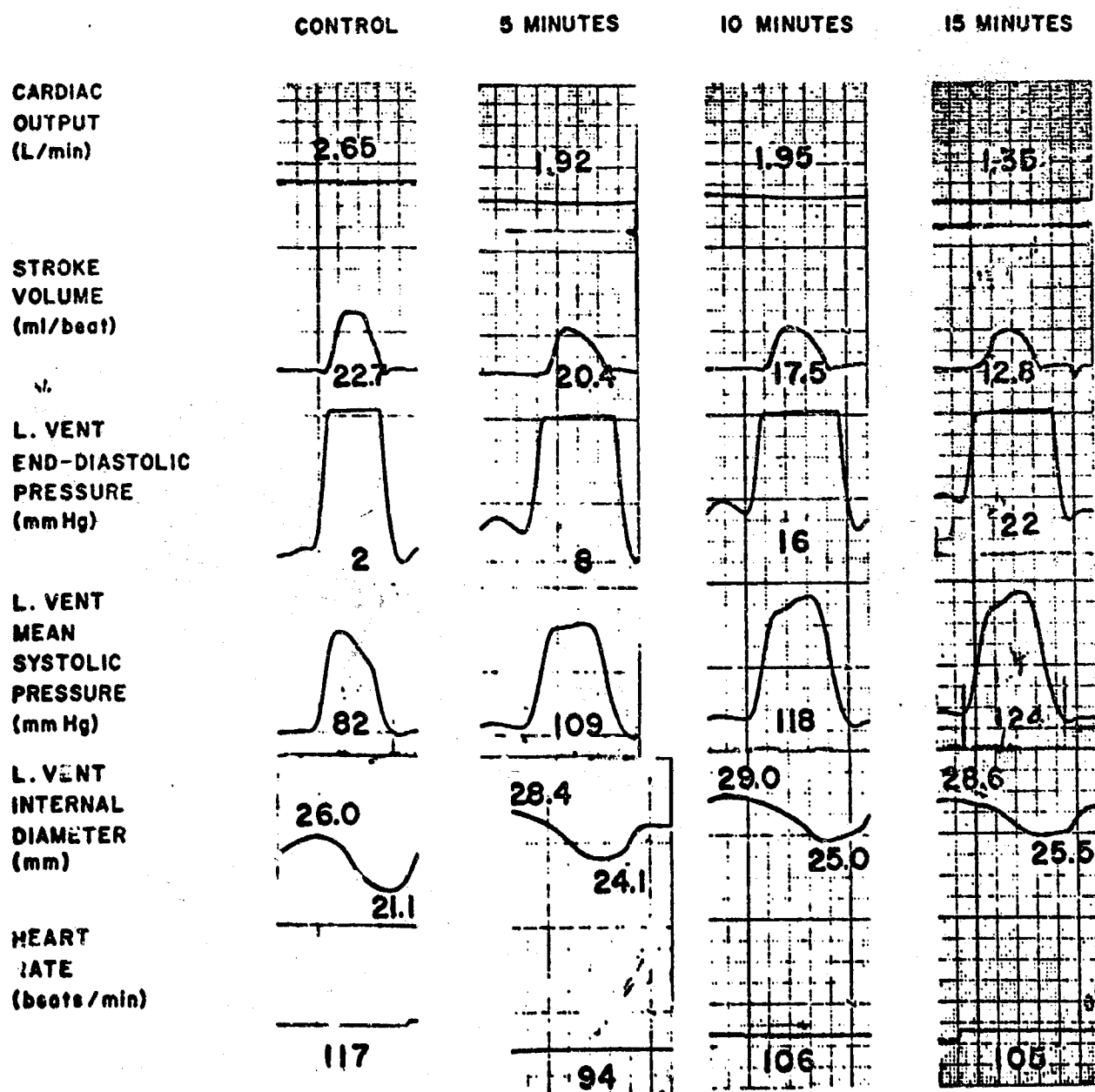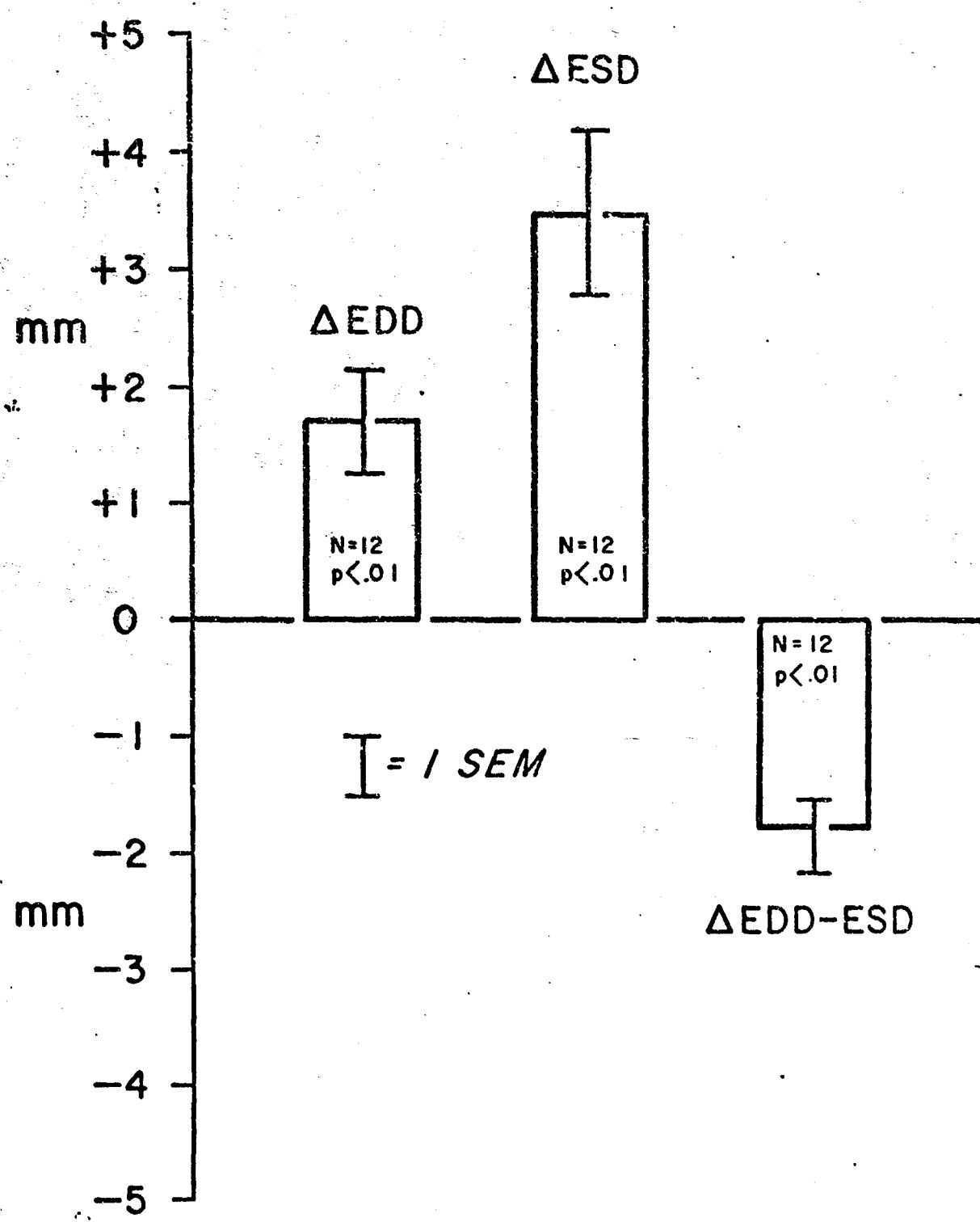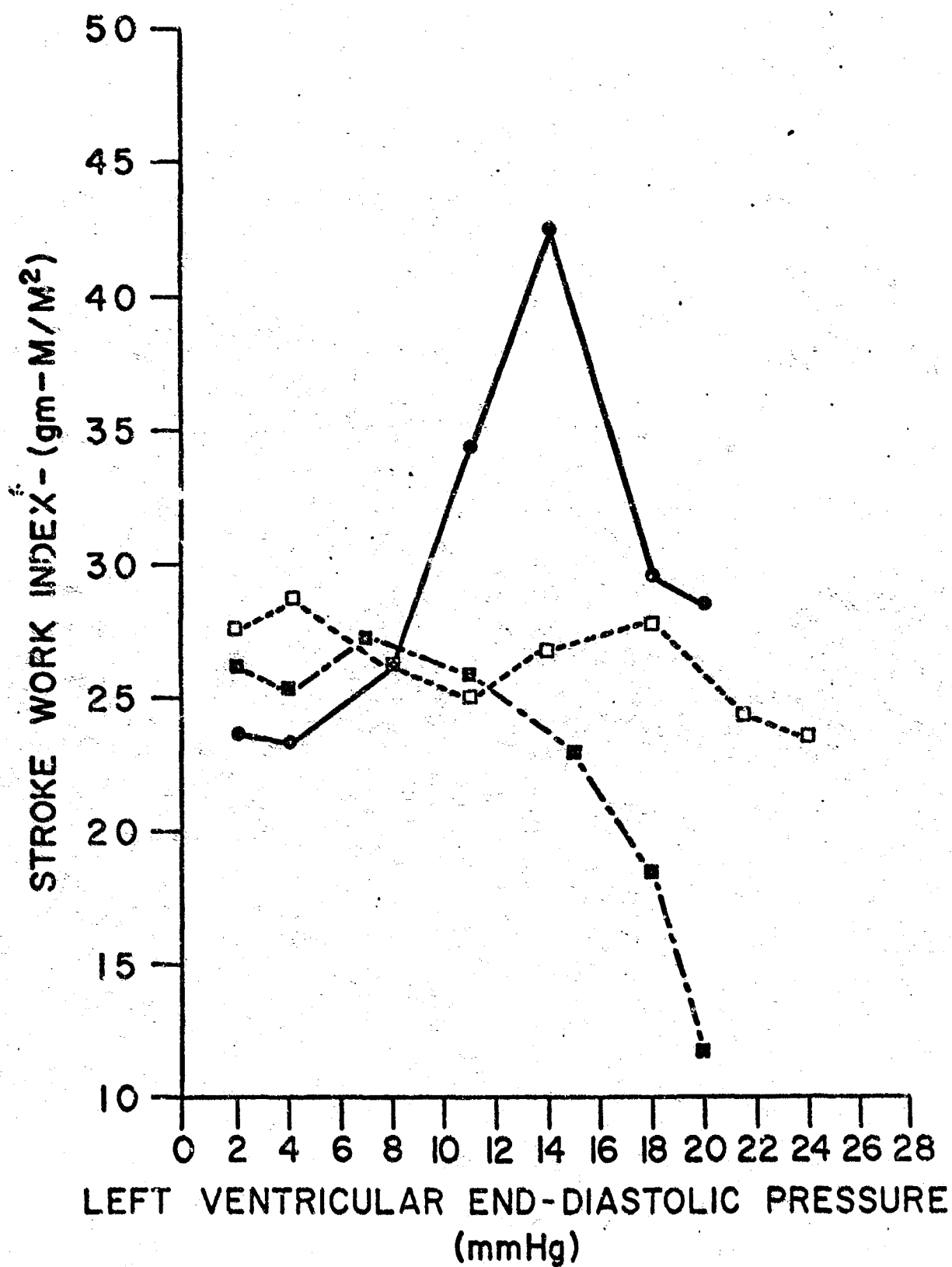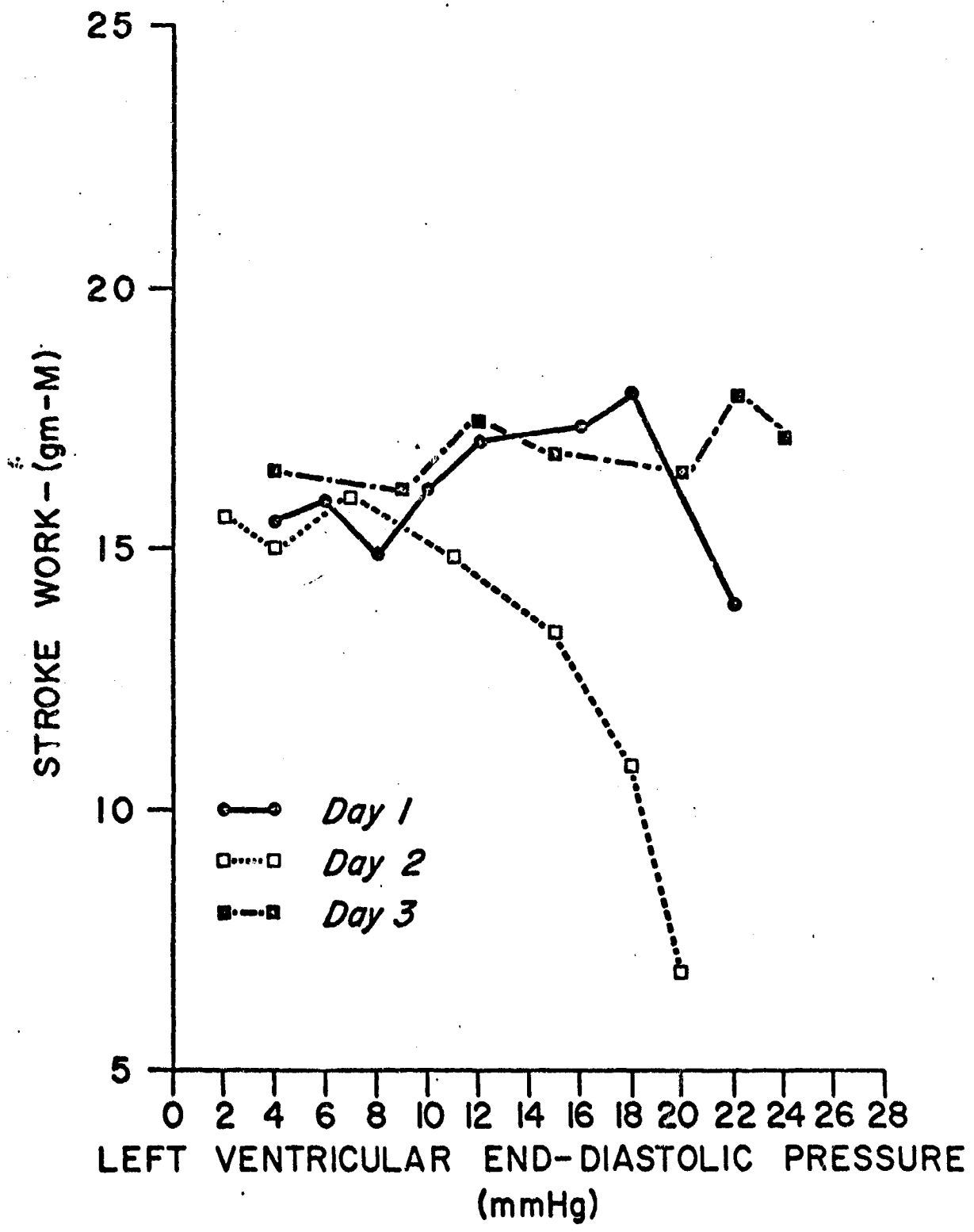
APPENDICES

A and B

Enclosed for use by the reviewers

Not a part of the manuscript itself

APPENDIX A   Hemodynamic data derived from twelve experiments in six conscious dogs during angiotensin infusion

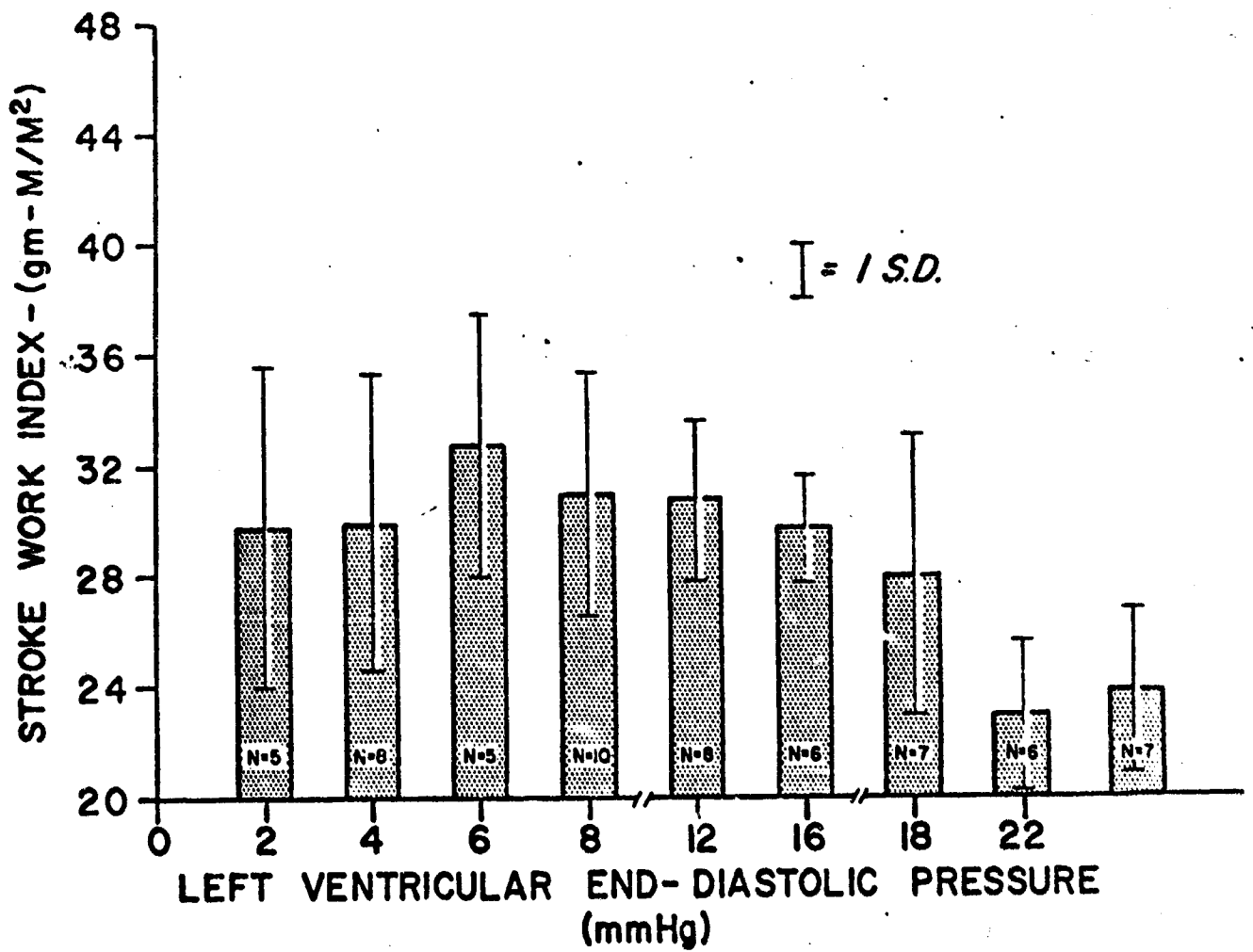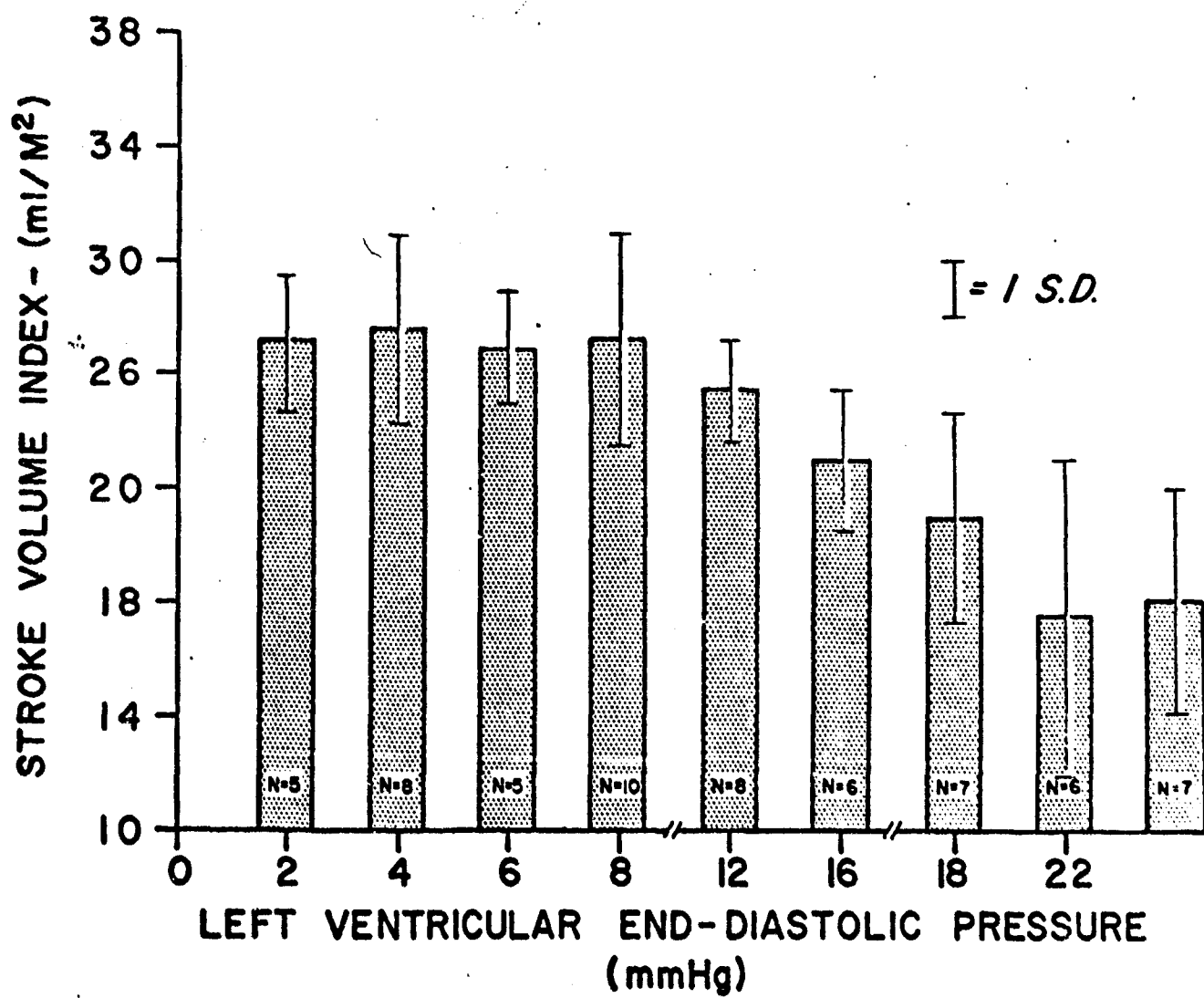| Animal Weight and Surface Area	Cardiac Output (L/min)	LV.Systolic mean Pressure (mmHg)	LV.end diastolic Pressure (mmHg)	Stroke volume (ml/beat)	Stroke volume Index (ml/beat-kg)	Stroke volume Index (ml/beat-m²)	Heart rate (beats/min)	Stroke work (gm-m)	Stroke work Index (gm-m/kg)	Stroke work Index (gm-m/m²)	Minute work (Kgm-m)	Minute work Index (Kgm-m/kg)	Minute work Index (Kgm-m/m²)
DOG 1-A	2.15	75	2	18.0	1.29	27.7	120	17.9	1.28	27.5	2.14	0.154	3.30
wt.= 14.0 kg	2.00	82	4	17.6	1.26	27.0	114	18.7	1.33	28.7	2.12	0.152	3.26
S.A.=0.65 m²	1.90	86	8	16.5	1.18	25.4	115	16.8	1.20	25.9	1.94	0.138	2.98
	1.93	90	11	16.3	1.16	25.0	118	16.3	1.16	25.0	2.07	0.148	3.10
	1.85	92	14	16.1	1.15	24.7	115	17.1	1.22	26.3	1.97	0.140	3.02
Experiment 1	1.80	103	18	15.5	1.11	23.8	116	17.9	1.28	27.5	2.33	0.166	3.57
	1.60	103	22	14.3	1.02	22.0	126	15.8	1.13	24.2	2.23	0.159	3.42
	1.68	105	24	13.9	0.99	21.4	120	15.5	1.11	23.9	1.87	0.133	2.87
	2.15	78	4	19.5	1.39	30.0	110	19.6	1.40	30.2	2.16	0.154	3.32
	.96	83	8	18.3	1.31	28.1	107	18.7	1.34	28.7	2.00	0.143	3.07
Experiment 2	2.13	92	12	18.8	1.34	28.9	113	20.5	1.47	31.6	2.32	0.166	3.56
	1.93	97	16	17.9	1.28	27.5	108	20.2	1.44	31.1	2.18	0.156	3.35
	1.85	105	16	15.4	1.10	23.7	120	18.6	1.33	28.6	2.24	0.160	3.44
	1.62	128	19	12.7	0.91	19.5	128	17.6	1.26	27.1	2.25	0.161	3.45
DOG 2-A	1.59	74	2	12.9	1.23	24.1	123	12.6	1.20	23.6	1.57	0.149	2.92
wt.= 10.5 kg	1.44	80	4	12.0	1.14	22.4	120	12.4	1.18	23.1	1.49	0.142	2.78
S.A.=0.536 m²	1.37	95	8	11.8	1.12	22.0	115	13.9	1.33	26.0	1.62	0.154	3.02
	1.53	105	12	14.5	1.38	27.1	105	18.3	1.75	34.2	1.93	0.184	3.60
Experiment 1	1.70	107	16	17.8	1.70	33.2	95	22.5	2.14	42.0	2.15	0.205	4.01
	1.65	112	18	12.6	1.18	23.4	117	15.9	1.51	29.6	1.85	0.177	3.46
DOG 3-A	2.40	95	2	22.2	1.23	28.8	108	28.1	1.56	36.5	3.04	0.169	3.94
wt.= 18.0 kg	2.56	111	5	21.3	1.18	27.7	116	27.8	1.54	36.1	3.34	0.186	4.34
S.A.=0.720 m²	2.45	106	6	21.1	1.17	27.4	116	28.7	1.59	37.3	3.33	0.185	4.32
	2.56	105	8	22.3	1.24	29.0	115	27.8	1.55	36.1	3.38	0.188	4.39
	1.80	110	12	20.0	1.11	26.0	90	26.7	1.48	34.6	2.40	0.133	3.12
Experiment 1	1.64	114	18	14.1	0.78	18.3	102	26.1	1.45	33.9	1.88	0.105	2.44
	1.28	117	20	11.1	0.62	14.4	115	18.6	1.03	24.2	1.69	0.094	2.20
	1.25	120	28	10.4	0.57	13.5	120	13.9	0.77	18.0	1.56	0.087	2.03
	2.65	82	2	22.7	1.26	29.7	117	24.7	1.37	32.1	2.89	0.160	3.38
	2.50	104	4	23.1	1.28	30.0	108	31.4	1.75	40.7	3.40	0.189	4.42
	2.24	108	6	20.7	1.15	26.9	102	28.7	1.59	37.3	3.11	0.172	4.06
Experiment 2	1.92	109	8	20.4	1.13	26.5	94	28.0	1.56	36.4	2.63	0.147	3.42
	1.95	112	12	18.7	1.04	24.4	104	25.4	1.41	33.0	2.65	0.148	3.46
	1.95	118	16	18.4	1.02	3.9	106	25.5	1.42	33.1	2.70	0.150	3.51
	1.35	124	22	12.8	0.60		115	14.5	0.81	18.9	1.75	0.097	2.27

APPENDIX A — Hemodynamic data derived from twelve experiments in six conscious dogs during angiotensin infusion

Animal Weight and Surface Area	Cardiac Output (L/min)	LV.Systolic mean Pressure (mmHg)	LV.end diastolic Pressure (mmHg)	Stroke volume (ml/beat)	Stroke volume Index (ml/beat-kg)	Stroke volume Index (ml/beat-m²)	Heart rate (beats/min)	Stroke work (gm-m)	Stroke work Index (gm-m/kg)	Stroke work Index (gm-m/m²)	Minute work (Kgm-m)	Minute work Index (Kgm-m/kg)	Minute work Index (Kgm-m/m²)
DOG 4-A wt = 10 kg S.A.=0.519 m² Experiment 1	1.32	93	6	12.6	1.26	24.3	105	14.9	1.49	28.7	1.57	0.157	3.01
	1.45	100	8	12.6	1.26	24.3	115	15.8	1.58	30.4	1.87	0.187	3.50
	1.22	108	10	13.6	1.36	26.2	90	18.1	1.81	34.7	2.63	0.163	3.14
	1.05	112	13	10.3	1.03	19.9	102	13.9	1.39	26.7	1.42	0.142	2.73
	1.00	121	16	9.8	0.98	18.9	102	14.0	1.40	27.0	1.43	0.143	2.75
	0.95	124	20	9.1	0.91	17.5	105	13.0	1.30	25.0	1.36	0.136	2.62
	0.90	130	22	8.3	0.83	16.0	108	12.3	1.22	23.5	1.30	0.130	2.50
	0.83	130	25	7.2	0.72	13.9	115	10.3	1.03	19.8	1.18	0.118	2.28
Experiment 2	1.40	98	8	14.0	1.40	27.0	100	17.1	1.71	33.0	1.71	0.171	3.30
	1.25	109	10	12.3	1.23	23.7	102	16.6	1.66	31.9	1.69	0.169	2.86
	1.40	118	14	15.6	1.56	30.0	90	22.1	2.21	42.5	1.99	0.199	3.83
	1.05	120	18	8.8	0.88	17.0	120	12.3	1.23	23.8	1.48	0.148	2.85
	0.93	128	20	8.8	0.88	17.0	105	12.9	1.29	24.9	1.36	0.136	2.62
	0.90	130	22	8.2	0.82	15.8	110	12.0	1.20	23.2	1.32	0.132	2.55
	0.93	132	24	8.1	0.81	15.6	115	12.0	1.20	23.0	1.38	0.138	2.65
DOG 5-A wt = 12.0 kg S.A.=0.588 m² Experiment 1	1.89	84	6	15.1	1.26	25.7	125	15.8	1.32	26.9	1.98	0.165	3.37
	1.83	83	8	14.6	1.22	24.8	125	14.9	1.24	25.3	1.87	0.155	3.16
	1.80	92	10	14.4	1.20	24.5	125	16.1	1.34	27.3	2.01	0.168	3.41
	1.56	101	12	14.2	1.18	24.2	110	17.2	1.43	29.2	1.89	0.157	3.21
	1.66	111	16	13.4	1.12	22.8	96	17.3	1.44	29.4	1.66	0.138	2.82
	1.22	111	18	14.2	1.18	24.2	86	18.0	1.50	30.5	1.55	0.129	2.63
	0.99	116	22	11.0	0.92	18.7	90	14.1	1.17	23.9	1.27	0.105	2.15
Experiment 2	1.78	78	2	14.8	1.23	25.2	120	15.3	1.27	26.0	1.84	0.152	3.12
	1.78	82	4	14.1	1.18	24.0	126	15.0	1.25	25.4	1.89	0.157	3.20
	1.52	96	7	12.9	1.08	21.9	118	16.0	1.33	27.1	1.89	0.157	3.20
	1.28	102	11	12.2	1.02	20.8	105	15.1	1.26	25.7	1.56	0.132	2.70
	1.21	107	15	11.0	0.92	18.7	110	13.8	1.14	23.4	1.52	0.125	2.57
	0.96	104	18	9.4	0.78	16.0	102	11.0	0.92	18.7	1.12	0.094	1.91
	0.59	105	20	5.4	0.45	9.2	110	7.0	0.59	11.9	0.77	0.065	1.31
Experiment 3	1.86	85	4	15.0	1.25	25.5	124	16.5	1.38	28.1	2.05	0.171	3.48
	1.40	94	9	14.0	1.17	23.8	100	16.2	1.35	27.5	1.62	0.135	2.75
	1.33	104	12	13.8	1.15	23.5	96	17.3	1.44	29.4	1.66	0.138	2.82
	1.08	107	15	13.0	1.00	20.4	90	16.6	1.39	28.3	1.49	0.125	2.55
	1.25	115	20	12.7	1.06	21.6	98	16.4	1.37	27.9	1.60	0.134	2.73
	1.30	123	22	12.7	1.06	21.6	102	17.4	1.45	29.7	1.78	0.148	3.03
	1.39	123	24	12.0	1.00	20.4	116	16.5	1.37	28.0	1.91	0.158	3.25

APPENDIX A

Hemodynamic data derived from twelve experiments in six conscious dogs during angiotensin infusion

Animal Weight and Surface Area	Cardiac Output (L/min)	LV.Systolic mean Pressure (mmHg)	LV.end diastolic Pressure (mmHg)	Stroke volume (ml/beat)	Stroke volume Index (ml/beat-kg)	Stroke volume Index (ml/beat-m²)	Heart rate (beats/min)	Stroke work (gm-m)	Stroke work Index (gm-m/kg)	Stroke work Index (gm-m/m²)	Minute work (Kgm-m)	Minute work Index (Kgm-m/kg)	Minute work Index (Kgm-m/m²)
DOG 6-A	1.80	86	4	18.0	1.50	30.7	100	20.1	1.67	34.1	2.61	0.217	4.43
Wt.= 12 kg	1.51	89	6	17.6	1.47	29.9	116	19.9	1.65	33.8	2.31	0.191	3.92
S.A.=0.588 m²	1.76	92	8	17.6	1.47	29.9	100	19.6	1.63	33.7	2.55	0.212	4.38
	1.00	98	12	14.3	1.19	24.3	70	16.3	1.36	27.7	1.43	0.119	2.43
Experiment 1	2.05	113	16	13.7	1.14	23.3	75	18.1	1.51	30.7	1.72	0.143	2.92
	1.00	118	18	13.7	1.14	23.3	73	18.6	1.55	31.7	1.73	0.144	1.95
	0.97	126	24	11.7	0.98	19.9	83	16.2	1.35	27.6	1.54	0.128	2.62
	1.67	75	4	18.0	1.51	30.6	92	17.5	1.46	29.8	1.96	0.164	3.34
	1.79	81	8	20.4	1.70	35.5	74	20.3	1.68	35.3	2.09	0.173	3.64
	1.76	86	10	18.7	1.55	32.1	94	18.8	1.56	31.9	2.14	0.177	3.04
Experiment 2	1.64	90	14	16.4	1.37	27.9	100	16.9	1.41	28.7	2.03	0.169	3.44
	1.33	106	16	15.6	1.30	26.5	85	19.1	1.58	32.5	2.01	0.165	3.41
	1.37	108	20	14.9	1.24	25.3	92	17.8	1.49	30.3	1.99	0.164	3.39
	1.37	112	22	14.6	1.22	24.8	94	17.9	1.49	30.4	2.04	0.169	3.67

APPENDIX B.   Ventricular dimensions in twelve experiments during angiotensin infusion

Group	EDD(mm)	ESD(mm)	EDD-ESD(mm)
DOG 1-A	21.5	16.4	5.1
	21.4	16.5	4.9
	22.1	17.3	4.8
	22.0	17.4	4.6
	22.1	17.7	4.4
Experiment 1	22.2	18.8	3.4
	22.2	19.0	3.2
	22.2	19.4	2.8
Experiment 2	21.7	16.7	5.0
	21.7	17.4	4.3
	22.0	17.6	4.4
	22.4	18.3	4.1
	22.0	18.4	3.6
	22.0	18.6	3.4
DOG 2-A	26.4	22.0	4.4
	26.6	22.4	4.2
	26.7	22.7	4.0
	27.0	22.3	4.7
	27.3	22.2	5.1
	26.5	22.3	4.2
Experiment 1	26.0	21.3	4.7
	26.2	21.6	4.6
	26.3	21.9	4.4
	26.4	21.7	4.7
	27.0	22.8	4.2
	26.8	23.9	3.7
	26.8	24.2	2.6
	26.7	24.4	2.3
DOG 3-A	26.0	21.1	4.9
	26.2	21.1	5.1
	26.8	22.0	4.8
Experiment 1	28.4	24.1	4.3
	29.0	25.0	4.0
Experiment 2	29.0	25.0	4.0
	28.6	25.5	3.1

APPENDIX B. Ventricular dimensions in twelve experiments during angiotensin infusion

	EDD(mm)	ESD(mm)	EDD-ESD(mm)
DOG 4-A	27.0	22.2	4.8
	27.2	22.4	4.8
	27.6	22.5	5.1
	27.5	22.9	4.6
	27.9	23.4	4.5
	28.2	23.9	4.3
	28.2	24.3	3.9
	28.1	24.9	3.2
Experiment 1	27.4	22.4	5.0
	27.6	22.7	4.9
	28.0	22.8	5.2
	27.8	23.8	4.0
	28.4	24.5	3.9
	28.5	25.0	3.5
	28.4	25.2	3.2
Experiment 2			
DOG 5-A	27.1	22.4	4.7
	27.1	23.7	3.4
	28.1	24.7	3.4
	30.1	26.7	3.4
	30.8	28.3	2.5
	30.5	27.8	2.7
	30.5	28.1	2.4
Experiment 1	27.9	24.1	3.8
	28.1	24.7	3.4
	28.8	26.0	2.8
	29.0	26.9	2.1
	29.9	27.4	2.5
	30.2	28.4	1.8
	30.2	28.6	1.6
Experiment 2	27.7	23.5	4.2
	29.2	25.4	3.8
	29.5	26.1	3.4
	29.5	26.4	3.1
	30.3	28.1	3.2
	30.2	28.0	3.2
	30.3	28.2	3.1
Experiment 3			

APPENDIX B. Ventricular dimensions in twelve experiments during angiotensin infusion

	EDD(mm)	ESD(mm)	EDD-ESD(mm)
DOG 6-A			
	28.4	23.7	4.7
	28.4	24.1	4.3
	28.6	24.5	4.1
	30.9	27.5	3.4
	30.6	27.7	2.9
Experiment 1	30.9	28.0	1.9
	31.2		0.7
	28.2	23.6	4.6
	28.5	23.6	4.9
	28.6	24.3	4.3
Experiment 2	30.4	27.0	3.4
	30.4	27.4	3.0
	30.4	27.6	2.8

Measurement of Left Ventricular Internal Diameter by Catheterization

by

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Running Head: Ventricular Diameter

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ABSTRACT

KARDON, M.B., O'ROURKE, R.A., PALMER, J., and BISHOP, V.S. Measurement of left ventricular internal diameter by catheterization.

Continuous recordings of left ventricular internal diameter were made in anesthetized dogs by retrograde aortic catheterization. The basic principle involved the measurement of ultrasonic transit time between two piezoelectric crystals mounted on a woven dacron cardiac catheter. The catheter could be manipulated so that it came to rest with a loop traversing the major chord of the left ventricular cavity parallel to the interventricular septum. Recordings obtained during the resting state, during norepinephrine infusion and during angiotensin infusion were similar to those previously obtained with implanted sonomicrometers.

Catheter   Ultrasound   Sonomicrometer

Ventricular Dimension

Hemodynamic measurements necessary to characterize myocardial function have been defined by the studies of Starling (9), Sarnoff (7), and Sonnenblick (8). However, these concepts cannot be readily applied to chronic animal studies or to clinical needs due to the lack of suitable techniques for the continuous measurement of cardiac dimensions. Thus, the pumping performance of the heart cannot be characterized in terms of its ability to perform as a muscle organ.

Recently, using surgical techniques, we have obtained continuous measurements of left ventricular internal diameter, blood pressure and flow in the conscious dog (1,4). This requires the implantation of sonomicrometer transducers on the endocardial surface of the left ventricle, a solid state pressure transducer in the apex of the left ventricle and an electromagnetic flow probe around the ascending aorta. These studies have provided important information regarding the interrelationships of these three variables and have emphasized the need for continuous measurements of left ventricular internal diameter and pressure by procedures which would not require a thoracotomy, and, could be readily used in research animals and in man.

In addition to the indicator dilution and angiocardiographic techniques, catheterization approaches for the measurement of left ventricular dimensions have been limited to electrical-mechanical systems (5,6) and the ultrasound pulse echo me   . The efficacy of electrical-mechanical systems is as yet unproven.

Carleton and Clark (2) have reported a ultrasound pulse echo technique for measuring the external diameter of the left ventricle from a catheter placed in the right ventricle. More recently a pulse

echo technique for topographical visualization of the left ventricle in the plane perpendicular to the catheter has been described by Eggeleton, et al (3). The catheter which has multiple piezoelectric crystals is made to rotate within the left ventricular cavity. This technique may be promising for analyzing asynchronous contractions in diseased hearts. The major difficulties in this approach lies in the effects of catheter movement and the rather sophisticated instrumentation needed for the measurement and analysis. The various limitations of the above techniques have led to the development of a catheter which measures the internal diameter and pressure of the left ventricle. This report describes a sonic transit time technique for the continuous measurement of left ventricular internal diameter.

Catheter Construction. A number of different catheter materials were tried and discarded, mainly because they either lacked the elastic properties necessary for this technique, were too stiff, or were too difficult to modify. Most of our success has been experienced with a woven dacron cardiac catheter.

Using a thin wall single lumen 8F woven dacron catheter, U.S.C.I. 5400 series, a 4 mm long semicylindrical section was cut from it along the preformed inner curve surface beginning 1 cm from the catheter tip. A second semicylindrical section of identical dimension was cut further down the catheter starting 8.5 cm from the tip. Consequently, with a bend made midway between these openings they could be made to face each other.

Two small rectangular pieces (3.5 mm x 2 mm) of ceramic piezo-electric material (resonant at 5 mHz) were cut from a 1" x 1" square,

using a carbide tipped scriber, by fracturing the ceramic along
the scribed line in a similar fashion to glass cutting. A length
of insulated single strand copper wire (Awg. 36) was soldered to
each face of both pieces of crystal material using a technique
similar to that used by Stegall, et al (10) and each pair was then
twisted together using a hand drill. A fish-wire of .015" bare
copper wire was advanced through the free lumen of the catheter
from the luer-lok end until it could be made to exit the farthest
(near the tip) window. Once outside the catheter the free ends of
one pair of signal leads, running to one rectangle of crystal, were
tied to the fish-wire; then it and the single pair of signal leads
were drawn down into the catheter until this tie point appeared oppo-
site the proximal window, which allowed us to tie the ends of the
second set of signal leads to the fish-wire also. Now, both pairs
of signal leads could be drawn through the catheter lumen until
they exited at its luer-lok fitting, at which time each pair
could be manipulated independently of the other. Starting with
the distal piezoelectric material each rectangle was affixed within
its respective window using a quick curing epoxy. The area above
each crystal surface was filled with the same epoxy material so that
the normal cylindrical surface of the catheter was restored.

In order to prevent the catheter from tearing at the corners
of each window when flexed backwards, we installed a 1 cm sleeve
of polyvinyl shrinkable tubing over each crystal and with the care-
ful use of heat it formed tightly over that area without melting
the catheter material itself. This technique, while preventing the

catheter from tearing, added somewhat to its overall stiffness in that area. A plug was formed at the luer-lok end by soldering each wire end to a contact pin and "potting" the four pins together. Connection to each crystal could then be made by this plug. Because of the manner in which this catheter contacts the endocardial surface it became obvious that while contact would be firm during ventricular systole, the catheter's innate resilience would be necessary to keep it firmly in contact with the ventricular surface during the more rapid phases of diastole since the catheter must not restrict the myocardial contraction. Thus, its maximum frequency response or stiffness is limited by this consideration.

Frequency response measurements were made in vitro by allowing the catheter to equilibrate for 20 minutes in a $37^{o}C$ water bath and then measuring its compliance simply by compressing it and allowing it to spring back while monitoring the sonomicrometer electrical output during its returning phase. By repeating this technique many times, the catheter's natural recovery rate over the range of dimensions expected in the left ventricle was found to be at least 1050 mm/sec. By placing a length of 22 gauge thin wall teflon tubing within the catheter lumen along with the transducer lead wires, the compliance of the catheter could be extended to at least 1180 mm/sec which was sufficient for most of our applications. In addition, the teflon lumen could be used for pressure measurements, albeit consⁱⁿ̱ᵉrably damped because of the small bore size. We found that further increases in compliance could be attained by advancing a .025" solid core guide wire (U.S.C.I.) inside the teflon bore, and

this, furthermore, could be done after catheter placement. In all cases the tip of the catheter was heat set into a gradual 130° bend from a point midway between the transducer sites.

The catheter was advanced under fluoroscopic control from a femoral artery cut-down site retrograde into the ascending aorta. At this point the catheter, because of its preset, would tend to form into a loop. We found that we were able to advance this loop, with point of flexure midway between sonomicrometer transducers, into the left ventricle. Once within the left ventricle, with the point of flexure resting at the apex of the heart, the tip of the catheter was free to spring outward and contact one ventricular wall while the main body of the catheter was in contact with the opposing wall (see Fig. 1). The animal was placed left side up with the sternum elevated about 30° from the horizontal and the catheter was positioned with fluoroscopic monitoring so that it came to rest with the loop traversing the major chord of the left ventricle parallel to the interventricular septum. In fact, the catheter exhibited a tendency to seek the largest dimension and because the transducers are radiopaque, it was easy to determine their exact position and the plane of orientation.

The electronic measurements were made using a technique essentially identical to that used by Stegall, et al (10). One transducer is made to produce a short burst of 5 mHz ultrasound while its opposing transducer senses the instant the sound wave reaches it. Knowing the velocity of sound in blood, the transit-time measurement is proportional to distance. The sonomicrometer

then gives an electrical output which can be calibrated to intraventricular dimension.

Animal Experiments. Four adult mongrel dogs weighing 15-18 kg were anesthetized with sodium pentobarbital. The right femoral artery was exposed so that the left ventricle could be catheterized by the retrograde approach with the cardiac dimension catheter. The left ventricular pressure was obtained either through the central lumen of the dimension catheter or through a second catheter passed retrograde into the left ventricle by way of the left carotid artery. Since the purpose of this initial study was to record left ventricular dimensions, little effort was made to assure that the lumen of the dimension catheter was unobstructed. Therefore, it was usually necessary to use a second catheter (7F) connected to a Statham P23Db pressure transducer for the measurement of the left ventricular pressure to insure a high fidelity response. The electrocardiogram was obtained from three subcutaneous needle electrodes. All signals were inscribed on an Electronics for Medicine recorder.

After the placement of the dimension and pressure catheters, a control recording was obtained over a 30 minute period. After this time isuprel (0.2 μg/kg-min) was administered intravenously using a Harvard Apparatus constant infusion pump. At the peak response, the isuprel infusion was stopped. When the measured variables had returned to the pre isuprel controlled state angiotensin (0.44 μg/kg-min) was infused intravenously until the peak left ventricular pressure reached a constant level at which time this infusion was stopped and

the parameters allowed to return to normal.

RESULTS

Figure 2 and 3 illustrates a recording of left ventricular
pressure, left ventricular transverse internal diameter and ECG
during control states and during the infusion of isuprel. The con-
tour of the transverse internal diameter recording as well as the
timing with respect to the left ventricular pressure and ECG is
similar to that previously obtained from implanted sonomicrometers.
The build up of the effect of isoproterenol is clearly shown in
this figure. At the peak effect the end diastolic and end systolic
diameter decreased. The average mean decrease was -6.4 ± 0.6 mm,
s.d. and -6.8 ± 1.4 mm respectively ($P < 0.01$)(Table 1). This re-
sponse of the transverse internal diameter clearly demonstrates how
an inotropic agent increases the performance of the heart.

With the angiotensin infusion the increase in left ventricular
systolic pressure was accompanied by an increase in the transverse
internal diameter. The elevated afterload increased the end systolic
diameter (+4.9 ± 0.8 mm, $P < 0.01$) more than the end diastolic diameter
(+2.8 mm ± 0.3 mm, $P < 0.01$), illustrating a decrease in shortening
of the myocardial fibers. Again the recordings in this figure are
similar to those obtained from the sonomicrometers implanted on the
endocardial surface of the left ventricle.

Although pressure recordings were not routinely made with the
dimension catheters used in this study, the catheters can be con-

structed so that the lumen will be adequate for high fidelity
pressure measurements. The catheter as demonstrated in this study,
provides a unique way of evaluating left ventricular internal di-
mensions of the heart on a beat-to-beat basis. In addition to the
general advantage of the ultrasound technique, the piezoelectric
crystals are radiopaque and thus allow for easy visualization of
the plane of measurement and the movement of the piezoelectric crystal
against the anterior and posterior endocardial surface. In all dogs
the number of premature ventricular contractions occurring during
the positioning of this catheter were similar to those obtained
during routine catheterization of the left ventricle and were
transient. Thus, this technique should be extremely valuable in
the assessment of left ventricular function in nonthoracotomized
animals and with refinement may be of value in diagnostic left heart
catheterization in patients with heart disease.

FOOTNOTES

The animals involved in this study were maintained in accordance with the Guide for Laboratory Animal Facilities and Care" as published by the National Academy of Sciences - National Research Council.

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TABLE 1

	HR b/min	EDD mm	ESD mm	LVSP mmHg	LVEDP mmHg
Control (average)	145±18 SEM	29.0±1.9	22.7±2.9	143±14	7.0±1.0
Isoproterenol (average)	225±11	25.0±2.0	15.9±3.2	150±17	4.0±2.0
Mean Difference	+81*	-6.4**	-6.8**	-7.4	-3.4
s.d.	±21	±0.6	±1.4	±11	±1.3
Control (average)	150±22	27.2±1.1	20.4±1.5	158±17	6.0±2.0
Angiotensin (average)	158±22	30.0±1.2	25.3±0.9	189±22	20.0±4.0
Mean Difference	+7.8	+2.8**	+4.9**	+31**	+14*
s.d.	±9.0	±0.3	±0.8	±5.0	±3.8

HR = heart rate, EDD = end diastolic diameter, ESD = end systolic diameter, LVSP = left ventricular systolic pressure, and LVEDP = left ventricular end diastolic pressure.

Isoproterenol rate of infusion = 0.2 μg/kg-min

Angiotensin rate of infusion = 0.44 μg/kg-min

Average ± = SEM

* P<0.05

** P<0.01

Significance tested by paired analysis

s.d. = standard deviation of the mean difference.

247

LEGENDS

Fig. 1.   Cutaway view of the left ventricle showing the dimension

catheter entering through the leaflets of the aortic valve

and resting in a plane parallel to the interventricular

septum.

Fig. 2.   Control recordings taken before isuprel infusion showing

electrocardiogram, left ventricular internal diameter and

left ventricular pressure. Note the presence of the

characteristic increase in ventricular diameter which is

synchronous with atrial contraction.

Fig. 3.   Taken during isuprel infusion decreases in both end diastolic

diameter and end systolic diameter are clearly discernable

as the diameter recording is scanned f: .. left to right.

Increased peak left ventricular pressure above control re-

cordings (Fig. 2) indicates the potent cardiac stimulating

effect of this beta-adrenergic sympathomimetic drug.
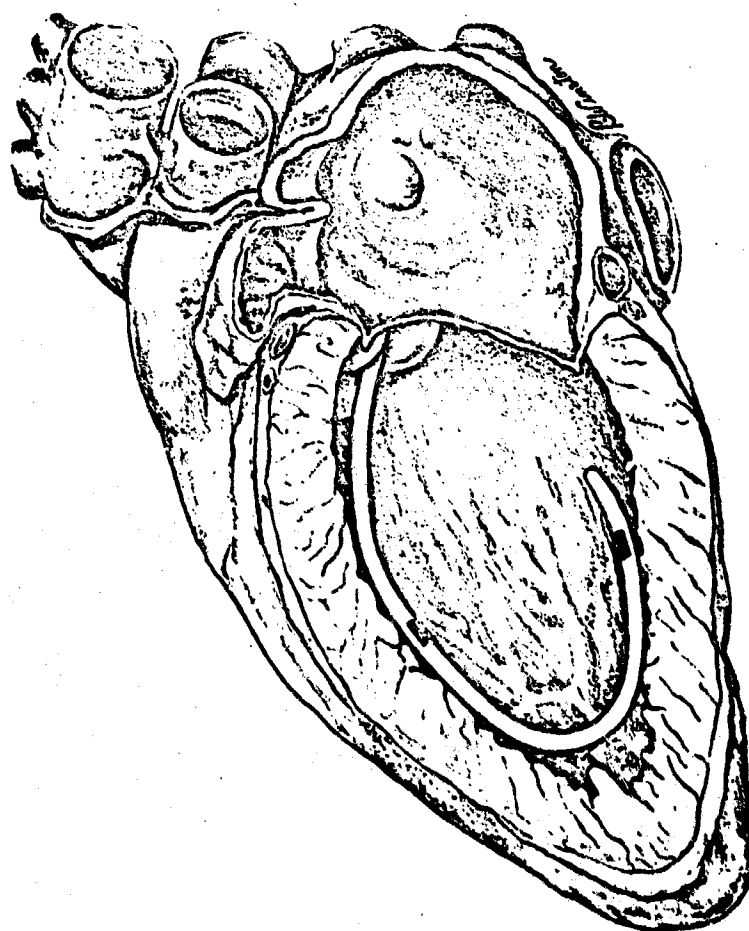
CONTROL

0.5 sec

26

20

INTERNAL DIAMETER (mm)

200

LEFT VENTRICULAR PRESSURE (mm Hg)

0

ISUPREL (5)

0.5 sec

26
20

INTERNAL DIAMETER (mm)

200
0

LEFT VENTRICULAR PRESSURE (mm Hg)

246
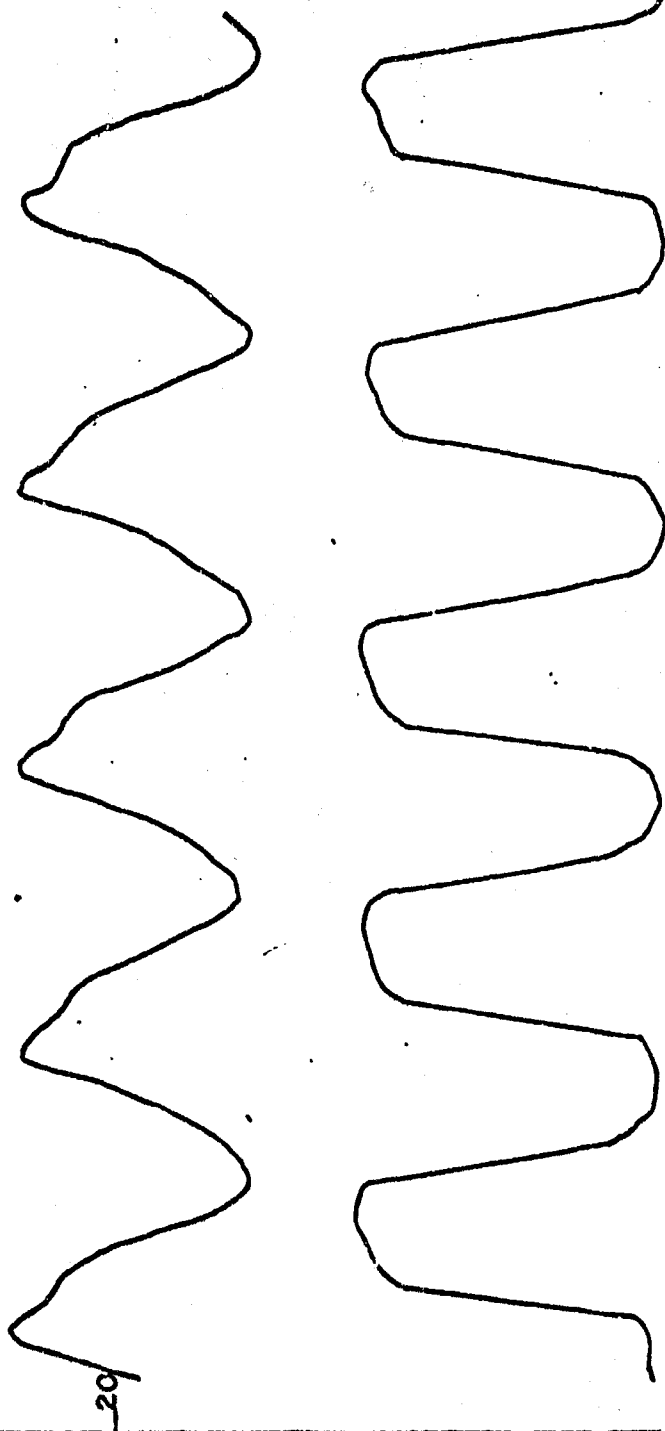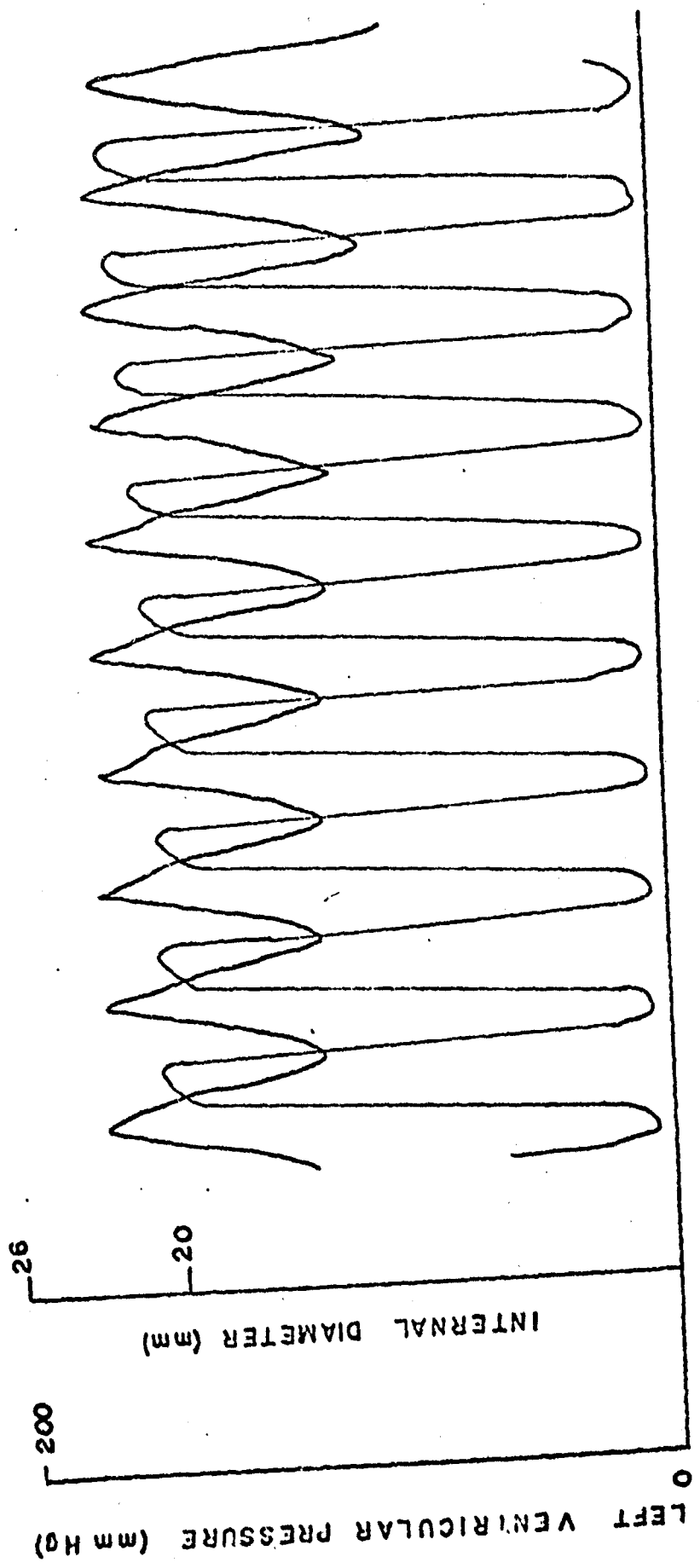
ACKNOWLEDGEMENTS

THE INTERACTION OF ACETYLCHOLINE AND NOREPINEPHRINE ON HEART RATE[1]

by

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ABSTRACT

Carrier, Gerald O. and Vernon S. Bishop. The interaction of
acetylcholine and norepinephrine on heart rate. J. Pharmacol. Exp. Ther.
The effects of acetylcholine and norepinephrine at 10^{-8} to 10^{-4} M were
obtained for isolated rabbit atria. The minimum negative chronotropic
response occurred at 10^{-7} M acetylcholine while 10^{-6} M produced a maximum
decrease in rate. Norepinephrine caused a maximum positive chronotropic
response at 10^{-4} M. The effects of fixed concentrations of norepinephrine
on the chronotropic response to acetylcholine and vice versa were deter-
mined. Acetylcholine (10^{-7}, 10^{-6}, 10^{-5}M) shifted the norepinephrine
curve to the right. Atropine (0.1 mg%) abolished the influence of acetyl-
choline. Norepinephrine (10^{-7}, 10^{-6}, __ M) increased heart rate above
control when 10^{-8} M acetylcholine was present. Propranolol (10^{-5} M) pre-
vented the influence of norepinephrine. As the concentration of
acetylcholine was increased, norepinephrine became less effective in
altering acetylcholine's chronotropic response. When both neurotrans-
mitters were present in equimolar concentrations, a pure cholinergic
effect was seen. For any norepinephrine/acetylcholine ratio, the effect
could not be expressed as the algebraic sum of the two separate effects.
Kinetic constants were estimated for norepinephrine alone and in the
presence of 10^{-7} M and 10^{-6} M acetylcholine. Vmax values were 64.5%, 65.7%
and 60.9% while the Ka values were 1.6×10^{-6} M, 4.7×10^{-6} M, and 4.5×10^{-5} M
respectively. The results of the present study suggest that during
vagal and sympathetic stimulation, the cholinergic system has the
greater influence on the chronotropic response of rabbit atria.

Running Title: Chronotropic Effect of ACh and NE

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The sinoatrial node receives its nerve supply from both the parasympathetic and sympathetic divisions of the autonomic nervous system. Under most conditions both sets of nerves are tonically active. Since some tonic activity usually exists in both divisions of the autonomic nervous system, a satisfactory quantitative description of the autonomic control must take into account the response to simultaneous activity in both the sympathetic and parasympathetic nerves (Levy and Zieske, 1969).

Hunt (1897) concluded that the change in heart rate resulting from simultaneous stimulation of the two sets of nerve fibers can be expressed as the arithmetical mean of the results of stimulating each set of nerve fibers separately. Rosenblueth and Simeone (1934) described mathematically the autonomic neural control of cardiac pacemaker activity. They stated that simultaneous excitation of the accelerator and decelerator nerves provoked a change in rate which cannot be expressed as the algebraic summation of the responses to separate stimulation. Stimulation of the vagus at various frequencies resulted in the same percent change in heart rate of the corresponding basal rate independent of the prevailing level of the sympathetic nerves and vice versa. Samaan (1935) observed that the antagonism between the cardio-accelerator and the cardio-inhibitory nerves on the rhythm of the ventricular muscle is not the algebraic sum of the two components, but that vagal activity had a more prevalent influence. Warner and Russell (1969) presented a mathematical model illustrating the effect of combined sympathetic and vagal

stimulation on the sinoatrial frequency in the dog. These results
and those obtained by Levy and Zieske (1969) are in agreement with
Samaan (1935). Recently Grodner et al., (1970) described the inter-
action of acetylcholine and norepinephrine on isolated rat atria. The
results obtained by these investigators were qualitatively in agreement with
our findings (Carrier and Bishop, 1970). However, the concentrations of
acetylcholine over the range employed by Grodner were one hundred times
the concentrations of norepinephrine, and this difference could be con-
sidered to have biased the results in favor of acetylcholine's effect.
These investigators (Grodner et al., 1970) stated that the concentra-
tions of acetylcholine (10^{-5}M to 10^{-3}M) employed to cause a slowing in
rate of the rat atrium were not unreasonable since the sinoatrial node
possesses a relatively high cholinesterase activity. However, Roberts
and Knojovic (1969) were able to demonstrate a slowing of the rat
atrium with acetylcholine in a range 100-fold less than that employed
by Grodner. There seems to be a discrepancy in the minimum dose of
acetylcholine required in order to cause a decrease in heart rate in
rat atrium. The concentrations of acetylcholine employed in the present
study are in agreement with Roberts and Knojovic (1969) and the decrease
in heart rate seen with the concentration range used in this study
are quantitatively similar to the results obtained by these investi-
gators.

The purpose of the present study was to investigate the inter-
action between acetylcholine and norepinephrine on the chronotropic
response in rabbit atria _in vitro_ so that a quantitative relationship

between the two neurotransmitters could be established. The concentration range was the same for both agents and all possible combinations were investigated.

METHODS

Albino rabbits of either sex and weighing approximately 2 pounds were used in this study. Animals were sacrificed by a blow to the head and were bled from the carotid artery. The hearts were rapidly excised and placed in oxygenated Ringer's solution. The spontaneously-beating right and left atria were dissected free and suspended in an organ bath containing 100 ml of Ringer's solution, pH 7.0 of the following composition: NaCl, 154 mM; KCl, 5.4 mM; $CaCl_2$, 2.4 mM; $NaCO_3$, 6 mM; and dextrose, 11 mM to one liter of double distilled deionized water. The organ bath was continuously oxygenated with 95% oxygen – 5% carbon dioxide and maintained at a constant temperature of $31^{o}C$ during the experiment. Immediately upon placing the spontaneously-beating atria in the bath, one gram of diastolic tension was applied. The preparations were allowed to equilibrate for one hour or until a constant heart rate and tension were maintained.

Drugs: Drugs used in this study were 1-norepinephrine bitartrate (Sigma Chemical Company) and acetylcholine bromide (Nutritional Biochemical Corporation). All drugs were solubilized in double distilled deionized water. Drug concentrations were calculated in terms of the salt and were prepared 30 minutes prior to each experiment.

Protocol: After the initial equilibration period, the influence of various concentrations of acetylcholine and norepinephrine alone

and in various combinations on the chronotropic response of isolated
rabbit atria was monitored with a Beckman Cardiotachometer and a
Type RM Dynograph. Dose-response curves were constructed, each curve
representing a different group of rabbit atria. After each exposure
of the atria to a certain concentration of the drug or drugs, the prepara-
tions were washed twice with Ringer's and allowed to reequilibrate to
baseline before subjecting them to additional exposures of the drug
or drugs. During the course of these experiments all atria prepara-
tions that developed arrhythmic activity were discarded. The follow-
ing dose-response relationships were obtained:

(a) Effects of acetylcholine on the chronotropic response

(b) Effects of norepinephrine on the chronotropic response

(c) Influence of various concentrations (10^{-7}M, 10^{-6}M, 10^{-5}M)
of norepinephrine on the normal acetylcholine heart rate
response

(d) Influence of various concentrations (10^{-7}M, 10^{-6}M, 10^{-5}M)
of acetylcholine on the normal heart rate response to
norepinephrine

(e) Interaction of acetylcholine and norepinephrine in equimolar
concentrations (10^{-7}M, 10^{-6}M, 10^{-5}M) on the chronotropic re-
sponse of spontaneously beating rabbit atria

In the experiments where the heart rate response was monitored after
the addition of various combinations of the agents used, the atria
were subjected to each of the agents separately, and time was allowed
for its full response to take place. Immediately after responding to

the first agent, the next drug was added to the bath. Addition of both agents simultaneously was done in a few of the experiments, but the final heart rate response showed no difference from the protocol employed.

Data Analysis: Changes in heart rate are expressed as percent change of the control heart rate after the equilibration period. Statistical analysis was performed according to Student's t test. The kinetic values presented were obtained by the use of Lineweaver-Burk plots; calculations were done on a Ollivetti 101 desk computer.

RESULTS

Influence of norepinephrine on acetylcholine's chronotropic response. When acetylcholine was added to the bath a negative chronotropic response occurred in the spontaneously-beating rabbit atria (Fig. 1). The minimum response was obtained with 10^{-7}M acetylcholine which caused an approximate 5% decrease in heart rate. In the presence of 10^{-8}M acetylcholine, all three concentrations (10^{-7}M, 10^{-6}M, 10^{-5}M) of norepinephrine caused an increase in heart rate above control level. The change in heart rate was comparable to that seen with these concentrations of norepinephrine alone (Fig. 2). However, 10^{-7}M norepinephrine had no effect in altering the negative chronotropic response to 10^{-7}M or greater concentrations of acetylcholine. Concentrations of 10^{-6}M and 10^{-5}M norepinephrine were effective in maintaining a heart rate above control until acetylcholine was present at concentrations of 10^{-6}M or greater. A few preparations were treated with 10^{-5}M propranolol which blocked the influence of norepinephrine.

NOT REPRODUCIBLE

Effect of acetylcholine on norepinephrine's positive chrono-
tropic response: Figure 2 illustrates the positive chronotropic re-
sponse to norepinephrine with the maximum heart rate response occurring
at 10^{-4}M norepinephrine. All three concentrations (10^{-7}M, 10^{-6}M, 10^{-5}M)
of acetylcholine resulted in the normal dose-response curve being
shifted to the right. However, when norepinephrine was present in
concentrations of 10^{-5}M and greater, 10^{-7}M acetylcholine did not sig-
nificantly alter the normal norepinephrine heart rate response. Acetyl-
choline (10^{-6}M) significantly altered norepinephrine's chronotropic
response until 10^{-3}M norepinephrine was present. At this concentration
of norepinephrine the maximum response obtained was not significantly
different ($P < 0.1$) from the normal maximum response. Acetylcholine
10^{-6}M and 10^{-5}M altered the normal norepinephrine dose-response rela-
tionship in such a manner that the same maximum response was obtained
but at higher concentrations. A few preparations were pretreated with
0.1 mg% atropine which prevented the influence of acetylcholine.

The kinetic values in Table 1 were obtained using a Lineweaver-
Burk plot. The Vmax value for norepinephrine alone and in combination
with 10^{-7}M and 10^{-6}M acetylcholine are not significantly different.
However, there is a significant difference in the Km values. This in-
dicates that acetylcholine competively antagonizes norepinephrine's
chronotropic response on isolated rabbit atria.

Interaction of acetylcholine and norepinephrine in equimolar con-
centrations: Figure 3 illustrates the chronotropic response of isolated

rabbit atria when norepinephrine and acetylcholine are present in equimolar concentrations. It is evident from this data that changes in heart rate when both neurotransmitters are present cannot be expressed as the mathematical sum of the two separate effects. Theoretically, when 10^{-6}M norepinephrine (which causes an approximate 43% increase in heart rate) and 10^{-6}M acetylcholine (which causes a 23% decrease in heart rate) are present, the resulting rate should be a 20% increase if the new heart rate can be expressed as the mathematical sum. Experimentally this was not the case. If norepinephrine is added first and then acetylcholine or vice versa, the resulting heart rate was a pure cholinergic response. There was no difference between the experimental data obtained when the two agents were present in equimolar concentrations and when acetylcholine was present alone at the same concentrations.

DISCUSSION

Several attempts (Rosenblueth and Simeone, 1934; Warner and Russell, 1969) have been made to develop a mathematical model of the autonomic neural control of the heart with particular reference to heart rate control. Such a model must take into account the influence of both the parasympathetic and sympathetic nerves and the interaction of these two systems since they are both tonically active. Levy and Zieske (1969), by electrically stimulating the right stellate ganglion and the left vagus trunk, concluded the interaction was such that the influence of a given level of sympathetic activity became progressively less pronounced as the level of vagal activity increased.

In the present study, the data clearly indicates that the vagus

transmitter (acetylcholine) has a greater affinity for the mechanism responsible for alterations in heart rate than does norepinephrine (sympathetic transmitter) when both transmitters are present. When the isolated atria are subjected to acetylcholine or norepinephrine, separately a slowing or acceleration in heart rate, respectively, occurred. In the presence of acetylcholine (10^{-8}M), norepinephrine at concentrations used in this study caused a significant increase in heart rate (Fig. 1) equivalent to that seen when these same concentrations of norepinephrine were added alone (Fig. 2). At this concentration of acetylcholine we can assume that there was virtually no cholinergic influence present. Therefore, one would expect to see a pure adrenergic response. Increasing the concentration of acetylcholine to 10^{-7}M or 10^{-6}M resulted in significantly higher doses of norepinephrine being required to obtain the same maximum response (Fig. 2) or reverse the slowing effect of acetylcholine (Fig. 1). In the presence of 10^{-5}M acetylcholine which caused an approximate 60% depression in rate, concentrations of norepinephrine at least 100-fold greater than that of acetylcholine were required to cause a slight reversal of acetylcholine's effect. We did not achieve maximum response with norepinephrine in the concentration range employed when studying the interaction with 10^{-5}M acetylcholine. This concentration of acetylcholine had a profound effect on the heart rate response. In fact, it resulted in complete arrest of several of the atrial preparations.

Quantitating the resultant change in heart rate when both
the sympathetic and parasympathetic nerve fibers are stimulated simul-
taneously, two different conclusions have been put forth. Hunt (1897)
stated that the resultant change in heart rate, when both sets of
nerve fibers are stimulated, reflects a mathematical summation of
the two separate effects. Rosenblueth and Simeone (1934), on the
other hand, concluded that the influence of each division of the auto-
nomic system is exerted independently of the other. According to
these investigators (Rosenblueth and Simeone, 1934), the change in
heart rate when both acetylcholine and norepinephrine are present
in the bath, reflects the order in which the substances were added.
Acetylcholine's effect on heart rate should be independent of the
presence of norepinephrine and vice versa. The present study does
not support either of these different ideas. When equimolar con-
centrations of both norepinephrine and acetylcholine are present,
a pure cholinergic response was obtained. No difference was seen
whether acetylcholine or norepinephrine was added to the bath first.
Also, when both substances were present in effective concentrations
the resultant change in heart rate could not be expressed as a
algebraic sum of the two separate effects.

The present data suggests a competitive type interaction be-
tween the two neurotransmitters changes in heart rate. Furchgott
et al., (1960) has reported a similar finding in the electrical
activity of stimulated guinea pig atrium; however, one must be cautious
in interpreting the competition between acetylcholine and norepinephrine

NOT REPRODUCIBLE

on the heart rate response. Since atropine prevented acetylcholine from interacting with norepinephrine and propranolol prevented nor-epinephrine from influencing acetylcholine, we can conclude that the cholinergic and beta adrenergic receptors are not the site of the drug interaction observed in these studies.

If one considers a hypothetical receptor (RX) which mediate: frequency changes in the heart, then the antagonism between nor-epinephrine and acetylcholine can be explained schematically as shown in Figure 4. In Figure , it is suggested that acetylcholine attaches to a cholinergic "atropine sensitive" receptor which effects RX in an unknown manner to cause an alteration in ionic conductance resulting in depression of pacemaker activity. Norepinephrine in a similar manner, first combines with a "propranolol sensitive" site resulting in changes occurring at RX which causes favorable ionic changes for the acceleration of heart rate. This model allows one to envision the involvement of a common system which mediates changes in rate and a site RX at which the two neurotransmitters can compete. The nature of RX is unknown at this time and the feasibility of its existence cannot be accepted without questions. The present study presents no evidence for the nature of RX or its existence, but the possibility that it does exist can explain the kinetic values obtained in the present study.

Changes in heart rate result from alteration of electrical activity in the sinoatrial region of the heart. The chief distin-guishing characteristic of the pacemaker region is that the membrane

of the pacemaker fibers is never completely at rest and always has
a tendency to depolarize (Draper and Weidmann, 1951; West, 1955). The
slow depolarization that occurs during diastole has been called the
"pacemaker potential" by Hutter and Trautwein (1956). It has been proposed
that the pacemaker potential results from time and voltage dependent
alteration in sodium and potassium conductance (Trautwein, 1963).

One explanation of the observation of the present study could
be that acetylcholine has a greater affinity than norepinephrine for
the mechanism responsible for changing potassium permeability.

Acetylcholine has been shown to affect the electrical activity
of the pacemaker fibers and it has been proposed that the effect
seen is due to a seléctive increase in potassium permeability (Hoffman
and Cranefield, 1960). Weidmann (1956) has suggested that a increased
permeability to potassium could inhibit an inward movement of sodium
during diastole, thus preventing complete depolarization from occurring.

After the application of epinephrine to pacemaker fibers the rate
at which pacemaker potentials develop is increased (Hoffman and
Cranefield, 1960). However, a decrease in potassium permeability has
not been demonstrated. It has been suggested that epinephrine acts
directly on the excitatory mechanism of the cell membrane, perhaps
by making available more sodium carrier (Hutter, 1957).

FOOTNOTE

( )

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Table 1

Estimated Menten Michaelis' constants for norepinephrine alone and in the presence of acetylcholine (10^{-7}M and 10^{-6}M).
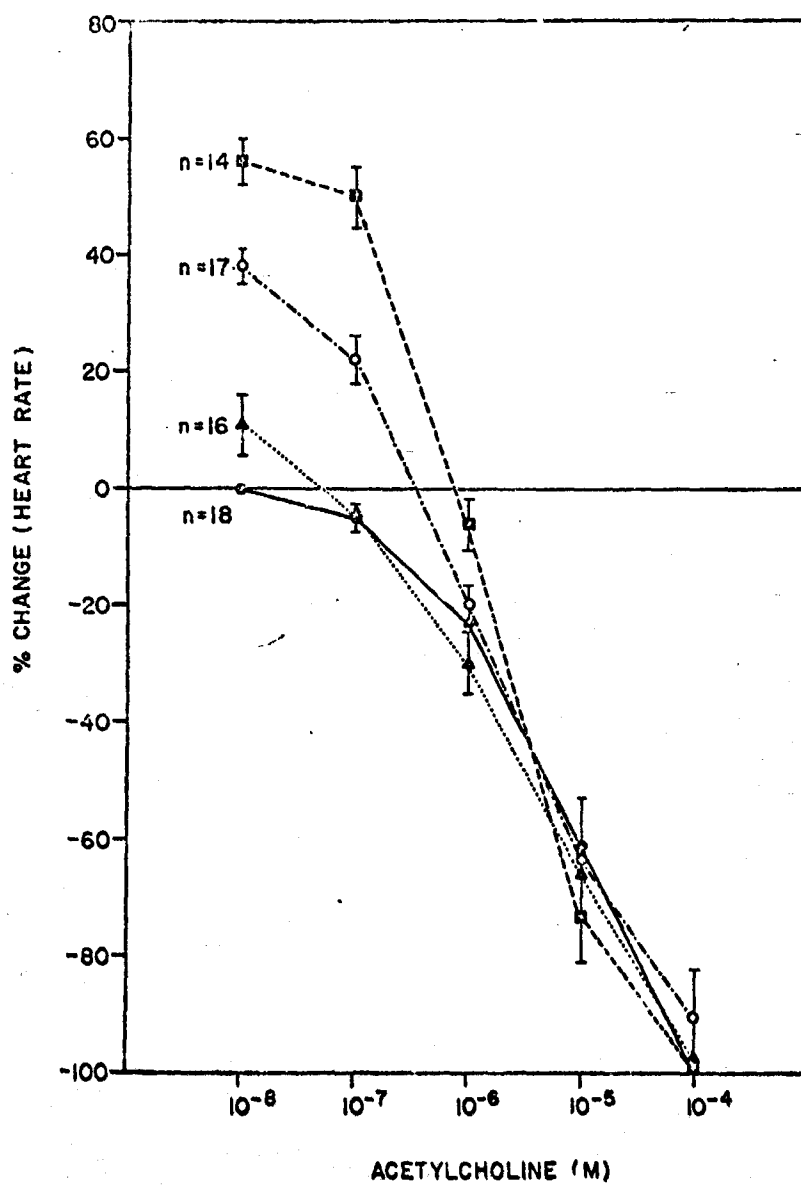
Sample	Vmax (% Change)	Km(M)
Norepinephrine	64.5	1.6×10^{-6}
Norepinephrine + 10^{-7}M Acetylcholine	65.7	4.1×10^{-6}
Norepinephrine + 10^{-6}M Acetylcholine	60.9	4.5×10^{-5}

LEGENDS

Figure 1: Influence of norepinephrine on acetylcholine's chronotropic responses in rabbit atria. ●——● illustrates the dose response relationship of acetylcholine. The effect of 10^{-7}M (▲.....▲), 10^{-6}M (0.-.-.0) and 10^{-5}M (▨-----▨) norepinephrine on acetylcholine's chronotropic response. All points represent the mean % change ± SEM.
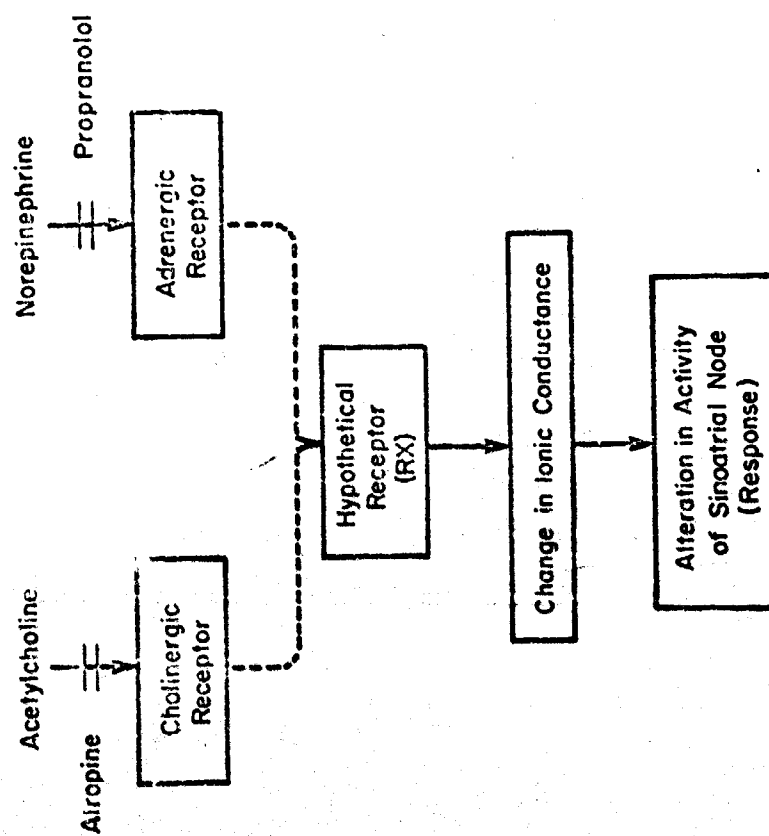
Figure 2: Influence of acetylcholine on norepinephrine's chronotropic responses in rabbit atria. ●——● illustrates the dose-response relationship of norepinephrine. The effect of 10^{-7}M (▲-----▲), 10^{-6}M (0-----0) and 10^{-5}M (▨-----▨) acetylcholine/norepinephrine's chronotropic response. All points represent the mean % change. The vertical bars represent the SEM.

Figure 3: Interaction of norepinephrine and acetylcholine in equimolar concentration. 0——0 illustrates the normal norepinephrine chronotropic response and ●——● illustrates acetylcholine's chronotropic response. ●.-.-.● represents the theoretical responses if the interaction of the two neurotransmitters can be expressed as the algebraic sum of the two separate effects. The experimental results are represented by △......△ (influence of norepinephrine in the presence of acetylcholine) and ▨------▨ (influence of acetylcholine in the presence of norepinephrine).

Figure with y-axis labeled "% CHANGE (HEART RATE)" ranging from -100 to 60, and x-axis labeled "NOREPINEPHRINE (M)" ranging from 10^{-8} to 10^{-3}. Curves labeled n=12, n=13, n=10, and n=14.

Norepinephrine

Propranolol

Adrenergic Receptor

Acetylcholine

Atropine

Cholinergic Receptor

Hypothetical Receptor (RX)

Change in Ionic Conductance

Alteration in Activity of Sinoatrial Node (Response)
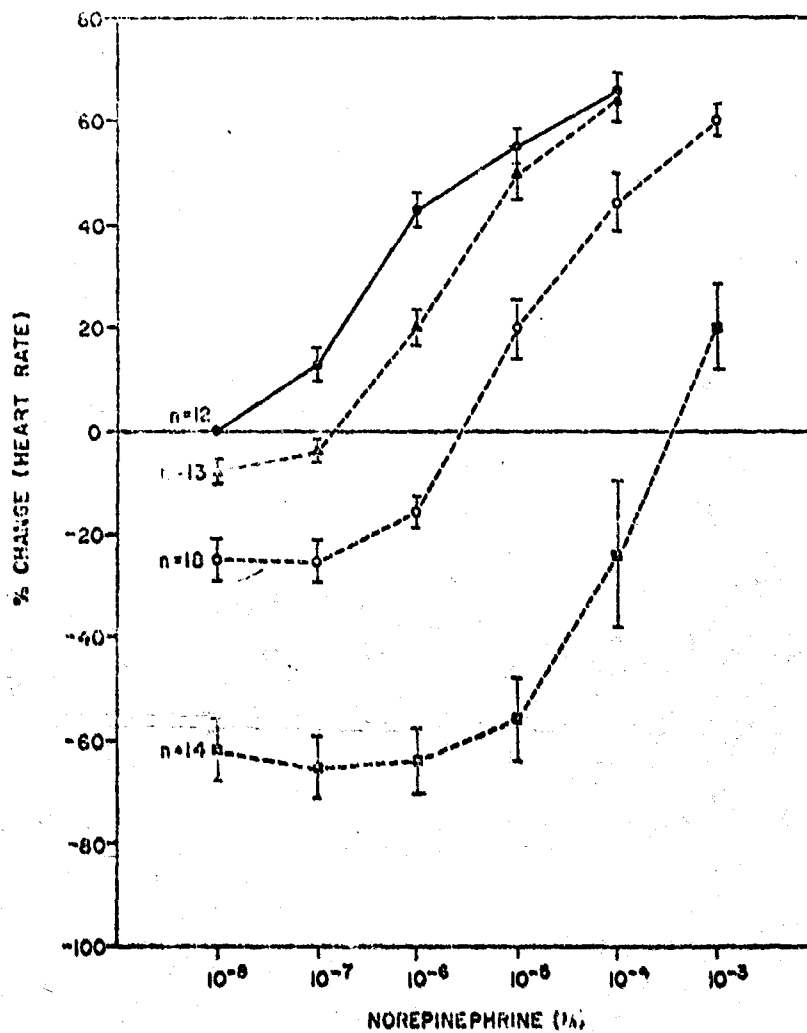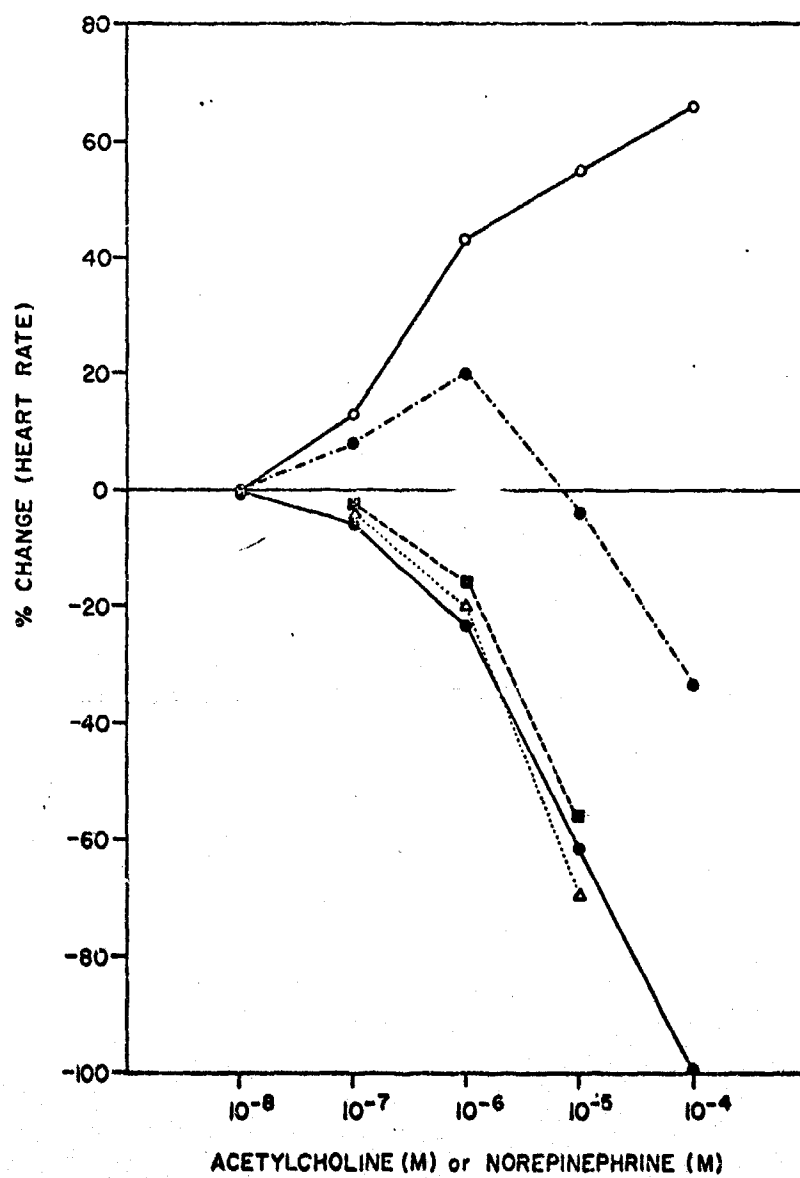
Studies in progress

a   Effects of atrial pacing (Fig. I & Table I)

b   Effects of increases in afterload (Tables
     II & III)

c   Effects of atrial pacing on increases in
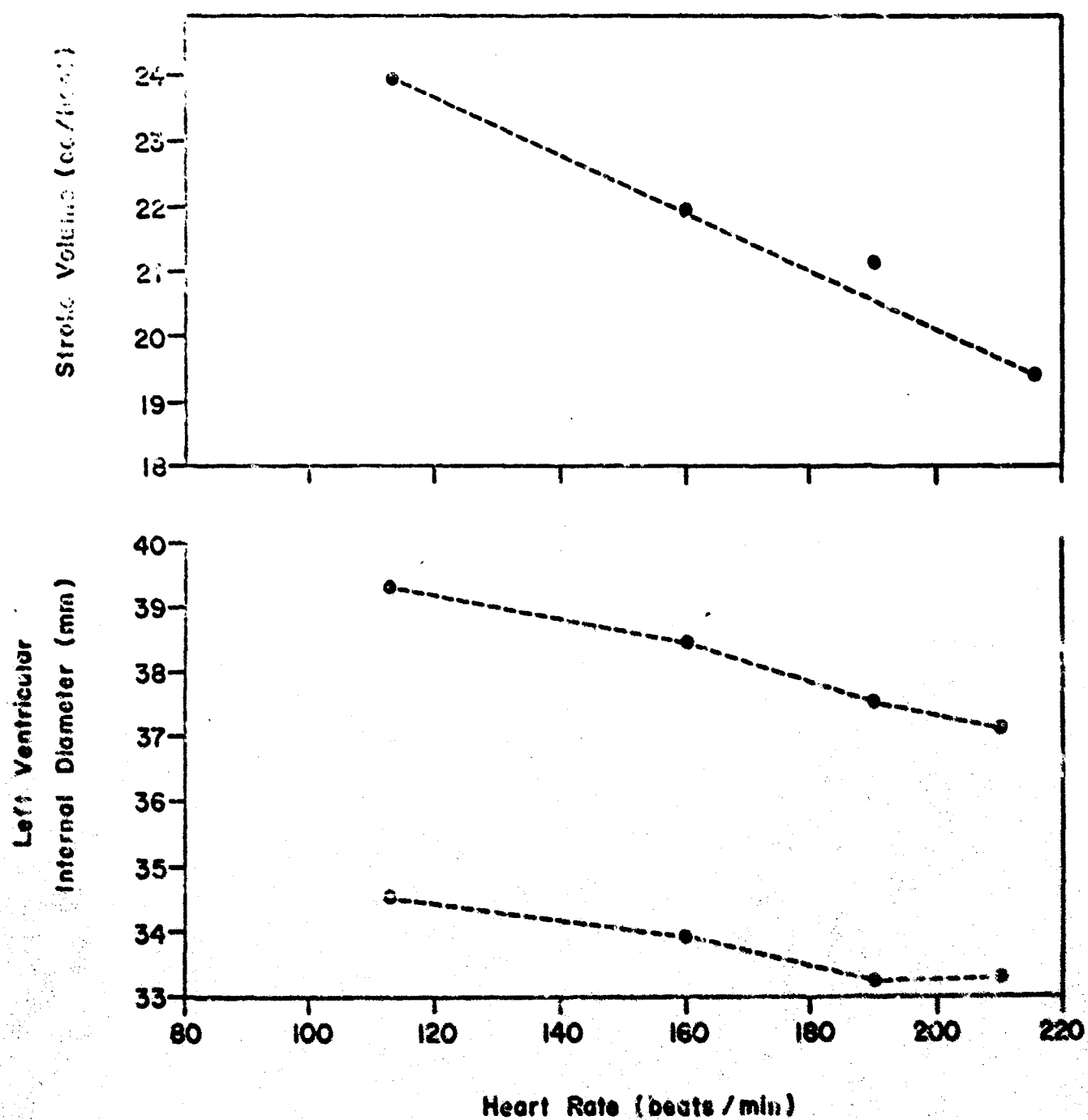   afterload (Tables II & III)

Table I: Cardiovascular parameters at rest

	HR (beat/min)	MAP (mmHg)	MLAP (mmHg)	LVMSP (mmHg)	$\frac{dp}{dt}$(max) mmHg/sec	$\frac{dF}{dt}$(max) cc/sec	$\frac{-dDs}{dt}$(max) mm/sec	$\frac{+dDd}{dt}$(max) mm/sec	SV cc/beat cc/kg-beat	EDD (mm)	ESD (mm)	(Δ)
DOG C-89	129	121	2.2	99	2588	5806	-58	66	0.93	24.6	20.9	3.
n =	6	6	6	6	6	6	6	6	6	6	6	
sd =	9	0.4	7.7	3.4	38	34	3.7	9.2	0.11	0.3	0.17	0.
DOG C-83	118	109	1.9	90	2408	2714	-49	88	0.96	36.4	31.9	5.
n =	7	7	7	7	7	7	7	7	7	7	7	
sd =	12	4.0	1.6	8.0	113	208	2.0	6.0	.13	0.3	0.1	0.
DOG C-84	113		4.3	88	2445	3624	-45	57	1.17	35.3	34.5	4.
n =			2	2	2	2	2	2	2	2	2	
sd =			0.2	2.5	30	35	1.0		0.12		0.1	
DOG 277	78		6.5	120	3300	4862	-50	62	0.84	38.5	32.8	5.7
DOG OVD	124		1.6	75	1980	6246	-52	48	0.69	33.2	29.6	3.6
n =	5		5	5	5	5	5	5	5	5	5	5
sd =	6.8		0.9	2.2	175	590	5.4	10.0	0.08	0.4	0.5	0.1
DOG J-10	118		1.8	136	2818	5318	-87	102	1.25	20.3	13.8	6.5
n =	2		2	2	2	2	2	2	2	2	2	2
sd =	1.4		0.1									
DOG 184	112		3.2	90	2712	4567	-50	55	0.82	37.6	33.7	3.8
n =	7		7	7	7	7	7	7	7	7	7	7
sd =	16.1		.6	14.2	48	212		6.4	0.10	0.5	0.5	0.4
DOG 32F	111			84	1970	4680	-38	0.68	0.78	31.0	27.5	3.5
n =	3			3	3	3	3	3	3	3	3	3
sd =	1.0			1.0	130	180	0	6.5	0.5	0.6	0.4	0.2
DOG 325	80		0.4	105	3117	6298	-62	0.75	0.98	35.2	28.5	5.7
n =	4		4	4	4	4	4	4	4	4	4	4
sd =	3.0		0.2	4.4	118	303	1.1	1.2	0.9	0.3	0.2	0.3
X̄	111	115	2.7	99	2593	4502	54	69.1	0.94	32.9	28.1	4.8
n =	9	2	8	9	9	9	9	9	9	9	9	9
	20	8	1.9	19	455	1192	17.1	17.1	0.18	6.6	6.4	1.3
	7	6	0.7	6	152	397	5.7	5.7	0.06	2.2	2.3	0.4

d̄ = mean difference, sd̄ = standard deviation of the difference, t = t value, df = degrees of freedom, n = number, SV = stroke volume, EDD = end diastolic diameter, ESD = end systolic diameter, ΔD = EDD-ESD(mm), $\frac{dP}{dt}$(max) = maximum deviation with respect to time of left ventricular pressure, mmHg/sec, $\frac{dF}{dt}$(max) = maximum deviation with respect to aortic flow, cc/sec, HR = heart rate, beats/min., MAP = mean arterial pressure mmHg, LVMSP = left ventricular mean systolic pressure, mmHg, and $\frac{-dDs}{dt}$(max) = maximum derivative with respect to time of left ventricular internal diameter during ejection, mm/sq, $\frac{-dDd}{dt}$(max) = maximum derivative with respect to time of left ventricular internal diameter during diastole, mm/sec.
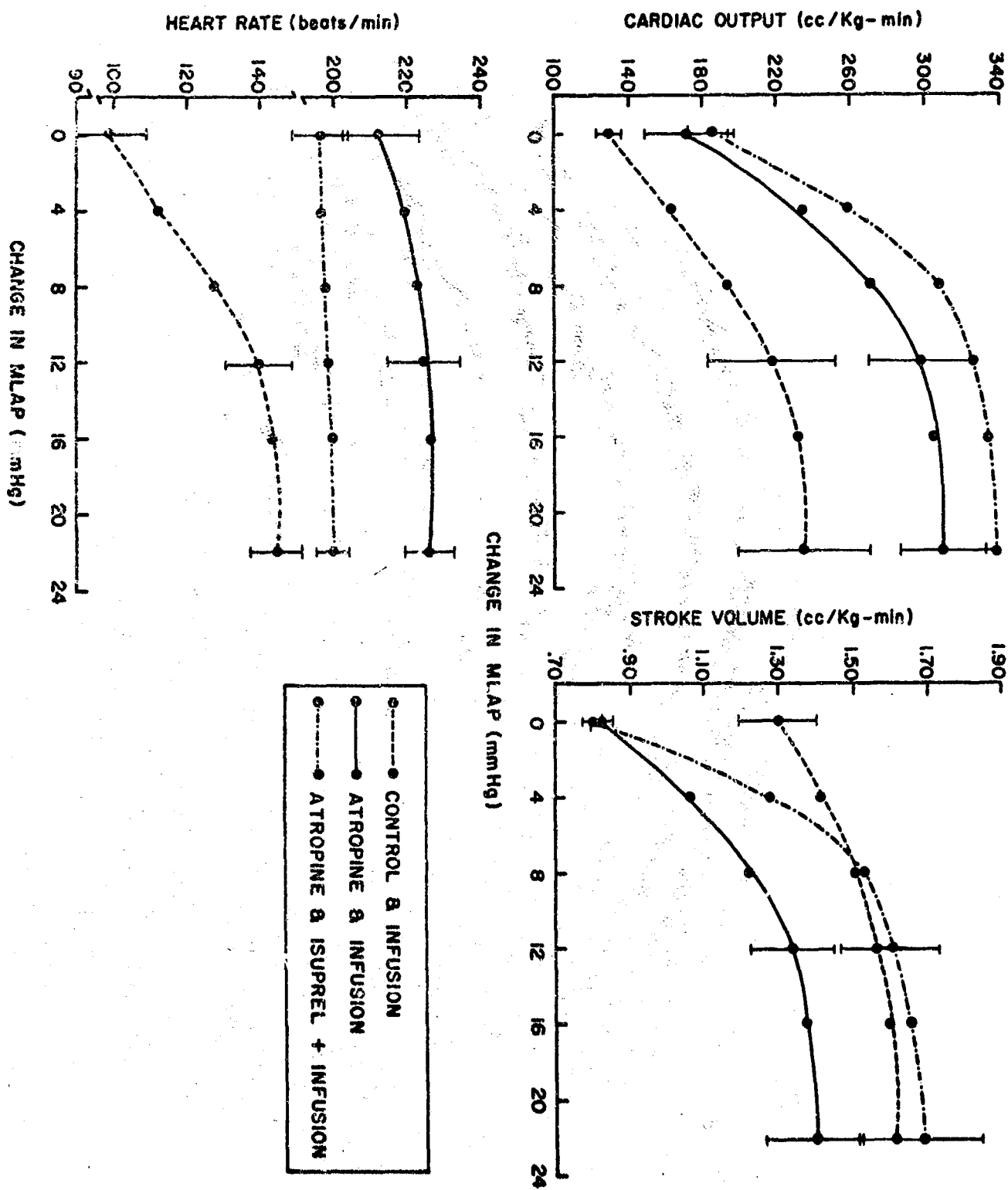
Table II: Mean changes in cardiovascular parameters with atrial pacing

	HR(beat/min)	MAP(mmHg)	MLAP(mmHg)	LVMSP(mmHg)	$\frac{dp}{dt}$(max) mmHg/sec	$\frac{dF}{dt}$(max) cc/sec	$\frac{-dD_s}{dt}$(max) mm/sec	$\frac{+dD_d}{dt}$(max) mm/sec	SV cc/beat cc/kg-beat	EDD(mm)	ESD(mm)	EDD-ESD (ΔD mm)
Experiment 1												
$\bar{d}$	48*	7.0	-0.4	-0.8	+139	-155*	0.8	12.9*	-6.20*	-1.6*	-0.62*	-1.0*
$s\bar{d}$	1.6	4.0	0.2	2.1	176	506	2.8	4.0	0.03	0.2	0.2	0.3
t	31.1	-1.8	1.5	0.8	0.8	3.2	0.3	3.2	6.3	6.4	3.2	3.9
df	6	1	7	7	7	7	7	7	7	7	7	7
n	7	2	8	8	8	8	8	8	8	8	8	8
Experiment 2												
$\bar{d}$	71*	-7.5	-0.9	6.2	-49	-852*	-3.5	+2.5	-0.25*	-2.1*	-1.0*	-1.3
$s\bar{d}$	3.8	12.5	0.4	7.5	34	152	7.0	7.2	6.04	0.11	0.2	0.5
t	.8	0.6	2.0	0.8	1.4	5.6	0.5	0.3	6.0	18.8	6.0	2.7
df	3	1	3	3	3	3	3	3	3	3	3	3
n	4	2	4	4	4	4	4	4	4	4	4	4
Experiment 3												
$\bar{d}$	96*		-0.22	0.6	92	-481	-6.6	-0.6	-0.32*	-2.9*	-1.0	-1.8*
$s\bar{d}$	2.8		0.9	2.2	80	246	5.1	9.94	0.06	0.3	0.5	0.6
t	23.8		0.2	0.3	1.1	2.0	1.3	0.1	5.1	9.1	2.0	2.9
df	4		4	4	4	4	4	4	4	4	4	4
n	5		5	5	5	5	5	5	5	5	5	5
Experiment 4												
$\bar{d}$	117*		-2.3*	0	-293	-1195*	-13	10.5	-0.44*	-3.1*	-0.9	-2.2*
$s\bar{d}$	4.2		0.7	3.24	97	64	5.7	20	0.09	0.5	0.3	.6
t	27.8		3.3	0	3.0	18.7	2.3	0.5	4.9	6.3	2.6	3.9
df	3		3	3	3	3	3	3	3	3	3	3
n	4		4	4	4	4	4	4	4	4	4	4

$\bar{d}$ = mean difference, $s\bar{d}$ = standard deviation of the difference, t = t value, df = degrees of freedom, n = number, SV = stroke volume, EDD = end diastolic diameter, ESD = end systolic diameter, ΔD = EDD-ESD(mm), $\frac{dp}{dt}$(max) = maximum derivative with respect to time of left ventricular pressure, mmHg/sec, $\frac{dF}{dt}$(max) = maximum deviation with respect to aortic flow, cc/sec. HR = heart rate, beats/min, MAP = mean arterial pressure mmHg, LVMSP = left ventricular mean systolic pressure, mmHg, and $\frac{+dD_s}{dt}$(max) = maximum derivative with respect to time of left ventricular internal diameter during ejection, mm/sec, $\frac{-dD_d}{dt}$(max) = maximum derivative with respect to time of left ventricular internal diameter during diastole, mm/sec.

Table III

	HR (beat/min)	MAP (mmHg)	MLAP (mmHg)	LVMSP (mmHg)	$\frac{dp}{dt}$(max) mmHg/sec	$\frac{dF}{dt}$(max) cc/sec	$\frac{-dDs}{dt}$(max) mm/sec	$\frac{+dDd}{dt}$(max) mm/sec	SV cc/beat cc/kg-beat	EDD (mm)	ESD (mm)	EDD-ESD (ΔDmm)
A.			Mean changes in cardiovascular parameters from control with increased afterload									
$\bar{d}$	14.8*	+17*	2.7*	21.*	107	-900*	-2.8	-5.8	-0.09*	1.1*	1.3*	-0.4
$s\bar{d}$	2.3	2.0	0.7	1.8	260	153	2.4	4.4	0.04	0.3	0.3	0.2
t	6.3	8.5	3.7	9.3	0.4	5.9	1.2	1.3	2.3	3.9	4.5	1.7
df	4	1	4	4	4	4	4	4	4	4	4	4
n	5	2	5	5	5	5	5	5	5	5	5	5
B.			Comparison of atrial pacing plus afterload with afterload alone									
$\bar{d}$	64*	9.0	-1.1	+1.4	78	86	-3.8	3.2	-0.13*	-1.2*	-0.4	-0.8*
$s\bar{d}$	1.9	11	0.5	2.4	173	120	2.6	4.6	0.04	0.26	0.22	0.2
t	33.7	0.8	2.4	0.6	0.5	0.7	1.5	-0.7	4.6	4.7	1.5	4.5
df	4	1	4	4	4	4	4	4	4	4	4	4
n	5	2	5	5	5	5	5	5	5	5	5	5
C.			Mean changes in cardiovascular parameters from control with increased afterload and atrial pacing									
$\bar{d}$	50*	26	1.6*	23.	185	815*	-6.8	-2.6	-0.27*	-0.1	+1.0*	-1.1*
$s\bar{d}$	3.6	13	0.5	1.2	111	111	3.2	7.6	0.02	0.3	0.2	0.2
t	14	2	3.6	18.4	1.6	7.3	2.1	0.3	15.4	0.3	3.9	5.7
df	3	1	4	4	4	4	4	4	4	4	4	4
n	4	2	5	5	5	5	5	5	5	5	5	5

$\bar{d}$ = mean difference, $s\bar{d}$ = standard deviation of the difference, t = t value, df = degrees of freedom, n = number, SV = stroke volume, EDD = end diastolic diameter, ESD = end systolic diameter, ΔD = EDD-ESD(mm), $\frac{dF_{(max)}}{dt}$ = maximum deviation with respect to time of left ventricular pressure, mmHg/sec, $\frac{dF_{(max)}}{dt}$ = maximum deviation with respect to aortic flow, cc/sec, HR = heart rate, beats/min., MAP = mean arterial pressure mmHg, LVMSP = left ventricular mean systolic pressure, mmHg, and $\frac{+dDs_{(max)}}{dt}$ = maximum derivative with respect to time of left ventricular internal diameter during ejection, mm/sec, $\frac{-dDd_{(max)}}{dt}$ = maximum derivative with respect to time of left ventricular internal diameter during diastole, mm/sec.

Mathematical description of ventricular
output curves

HEART RATE (beats/min)

CARDIAC OUTPUT (cc/Kg-min)

STROKE VOLUME (cc/Kg-min)

CHANGE IN MLAP (mmHg)

CHANGE IN MLAP (mmHg)

CONTROL & INFUSION
ATROPINE & INFUSION
ATROPINE & ISUPREL + INFUSION

Evaluation of the interaction of
propranolol and digitalis

MEAN INDIVIDUAL CHANGES IN THE MEASURED VARIABLE OF LEFT VENTRICULAR FUNCTION

	% HR	% SV	% EDD	% ESD	% LVPSP	dP/dt
Ouabain*	-11.9±5.8	$6.13±1.9^b$	0.3±1.3	-2.0±1.7	6.3±4.2	$18.8±6.4^a$
	(6)	(6)	(6)	(5)	(4)	(6)
Ouabain + Propranolol*	$-18.8±6.8^a$	$4.3±2.9^b$	3.3±1.1	1.9±1.5	1.8±4.3	4.0±4.4
	(6)	(6)	(6)	(6)	(4)	(6)
Ouabain + Propranolol**	$-10.1±3.2^b$	-1.0±2.4	-0.5±3.5	$3.8±1.4^a$	$-3.1±1.1^b$	$-13.1±2.2^e$
	(8)	(8)	(8)	(8)	(6)	(8)
Propranolol*	+1.3±5.0	$-4.82±1.8^a$	1.5±1.7	$5.72±1.1^d$	2.8±3.6	$-17.1±2.5^e$
	(6)	(5)	(6)	(6)	(4)	(6)
Propranolol + Ouabain*	-8.2±5.3	-6.2±2.9	-1.7±1.3	0.2±0.9	-1.5±2.5	-2.7±3.2
	(6)	(5)	(6)	(6)	(4)	(6)
Propranolol + Ouabain**	$-9.3±3.3^a$	0.3±3.8	$-3.1±1.2^a$	$-5.2±1.3^c$	-3.9±3.6	-2.4±19.7
	(6)	(6)	(6)	(6)	(4)	(6)

HR = Heart Rate   SV = Stroke Volume   EDD = End Diastolic Diameter   ESD = End Systolic Diameter

LVPSP = Left Ventricular Peak Systolic Pressure   F = Flow   * Control   ** Ouabain   *** Propranolol

^{a} P<0.05 ^{b} P<0.025 ^{c} P<0.01 ^{d} P<0.005 ^{e} P<0.001   ± SEM

AVERAGE MEASUREMENTS OF LEFT VENTRICULAR FUNCTION

Resting Conditions	HR(b/min)	SV(cc/b)	EDD(mm)	ESD(mm)	LVPSP(mmHg)
Control	112.8±5.7 (12)	20.0±1.4 (11)	29.0±1.5 (12)	23.3±1.5 (12)	129.6±8.9 (8)
Propranolol	112.0±6.1 (6)	19.9±2.2 (6)	29.0±2.2 (6)	24.1±2.3 (6)	135.0±8.9 (4)
Propranolol + Ouabain	100.9±4.9 (6)	20.3±2.7 (6)	28.2±2.3 (6)	23.0±2.4 (6)	130.1±8.0 (4)
Ouabain	103.3±5.8 (8)	23.0±1.7 (8)	32.1±5.8 (8)	26.8±2.7 (8)	138.5±14.6 (6)
Ouabain + Propranolol	93.4±6.4 (8)	23.0±1.8 (8)	33.0±2.4 (8)	27.6±2.7 (8)	135.2±37.2 (6)

HR = Heart Rate

SV = Stroke Volume

EDD = End Diastolic Diameter

ESD = End Systolic Diameter

LVPSP = Left Ventricular Peak Systolic Pressure

± = SEM

CENTRAL NERVOUS SYSTEM

Ronald D. Huffman

Air Force Progress Report

Central Nervous System - Synaptic Transmission in the Central Nervous System

A. Study of the effects of psychotomimetic and convulsant drugs on spinal

and supraspinal inhibitory mechanisms.

1. Progress Report

During the past twelve months, we have been studying the effects of the

psychotomimetic drugs on cerebellar disfacilitation and reticular inhibition

of the segmental monosynaptic potentials. The results of our experiments

(Figures 1 - 12) demonstrate a marked contrast in the sensitivity of these

two inhibitory systems to the action of the psychotomimetic drugs. So far,

we have studied the effects of LSD, mescaline and bufotenine on both types

of supraspinal inhibition of the segmental monosynaptic action potentials.

In addition, the effects of these drugs on reticular inhibition of the mono-

synaptic patellar reflex have been studied in numerous experiments.

Cerebellar disfacilitation of both extensor and flexor monosynaptic

potentials was completely blocked with intravenous injections of LSD

(Fig. 1, 2, 3). After 100-200µg/kg of LSD, the segmental monosynaptic

potentials were no longer inhibited as a result of cerebellar stimulation;

in fact, a pronounced facilitation of the segmental potentials was observed

with cerebellar stimulation following LSD (Fig. 1C, 2C, 3D). Equivalent

doses of LSD had no effect on the reticular inhibition of the segmental

monosynaptic action potentials or the monosynaptic patellar reflex (Fig. 9).

Similar results were obtained with bufotenine and mescaline on

cerebellar disfacilitation (Fig. 4, 5). Like LSD, these drugs completely

eliminated the cerebellar disfacilitation of the segmental monosynaptic potentials and revealed an underlying facilitation (Fig. 4B, 5C). The resistance of reticular inhibition to bufotenine and mescaline is illustrated in Figures 10 and 6, respectively. Doses of bufotenine 2 to 3 times that required to block cerebellar disfacilitation of the segmental monosynaptic potentials had no effect on reticular inhibition of these potentials or of the patellar reflex. These higher doses of bufotenine markedly depressed the segmental monosynaptic potentials and the patellar reflex (Fig. 10). The dose of bufotenine that depresses the cerebellar disfacilitation ($500\mu g/kg$) is also the dose that produces the abnormal somatomotor effects in conscious intact cats.

Psilocybin had no effect on reticular inhibition of the monosynaptic patellar reflex (Fig. 8A) or of the polysynaptic linguomandibular reflex (Fig. 8B). It did, however, produce a marked potentiation of the linguo-mandibular reflex. Studies on the effects of psilocybin on cerebellar disfacilitation and reticular inhibiton of the segmental monosynaptic action potentials are being conducted at the present time.

Some preliminary results from studies with Δ^1-tetrahydrocannabinol (THC), the active principle in marihuana, and Ditran, an anticholinergic glycolate ester (also a hallucinogenic compound) indicate that these psychotomimetic drugs have a depressant effect on the reticular inhibitory system. THC completely blocked reticular inhibition of the segmental monosynaptic potentials (Fig. 7). The reticular inhibition of these potentials was replaced by a marked facilitation (Fig. 7B, C). Ditran similarly blocked reticular inhibition of the monosynaptic pat llar

reflex in three studies (Fig. 11, 12). The effects of these drugs on cerebellar disfacilitation of the segmental monosynaptic potentials are presently being investigated.

It is possible that some of the psychotomimetic drugs may exert a direct depressant effect upon the inhibitory Purkinje neurons of the cerebellum and thus depress the functioning of these neurons. This would prevent the Purkinje neurons from exerting their normal inhibitory action on their target neurons -- the tonically active, excitory neurons of the deep cerebellar nuclei (fastigial, dentate, interpositus) and the lateral vestibular nuclei (Deiter's nucleus).

Deiter's nucleus is a major source of descending excitatory input to the spinal motoneurons and the activity of Deiter's neurons is under the direct control of the inhibitory Purkinje neurons of the cerebellum. Any depression of Purkinje neuronal activity by the psychotomimetic drugs would reduce the cerebellar disfacilitation of the segmental monosynaptic potentials and at the same time give rise to a facilitation of the uninhibited potentials (Fig. 1C; 2C).

Another possibility is that the psychotomimetic drugs are interfering with the release or action of the transmitter released by the Purkinje cells to inhibit Deiter's neurons. Recent experimental studies suggest that gamma aminobutyric acid (GABA) is the transmitter released by the cerebellar Purkinje cells to inhibit Deiter's neurons (Curtis, Duggan and Felix, 1970; Obata, Ito, Ochi, and Sato, 1967; Obata, Otsuka and Tanaka, 1970). GABA has also been implicated as the transmitter involved in producing the primary afferent depolarization that is presumably associated with presynaptic

with bipolar concentric electrodes. One msec-square wave pulses were applied to the RF (0.5-7V; 100 c/s) and to the cerebellum (2-12V, 150-200 c/s) for 5-15 sec. Gallamine triethiodide was administered to prevent excessive movements during stimulation, and the animals were artificially respired. Carotid blood pressure was monitored and body temperature was maintained at $37 \pm 1°C$.

Bicuculline was administered slowly in small doses (0.25-0.5mg/kg) and the total dose given was recorded as an accumulative dose for a given period. Drug effects were usually not recorded until 5 or 10min after the injection to minimize the potential effects of blood pressure changes.

Bicuculline suppressed both presynaptic and cerebellar inhibition (Fig. 13, 14, 15) of the segmental monosynaptic action potentials, but had no effect on direct or reticular inhibition (Fig. 15, 16, 17) of these same potentials. There was no observed difference in the effects of bicuculline on these inhibitions of extensor (quadriceps) and flexor (biceps semitendinosus) monosynaptic action potentials. Figure 17 illustrates a typical experiment. The two curves describe the change in amplitude of the quadriceps monosynaptic action potentials as a function of time following stimulation of the biceps semitendinosus nerve. The effects of reticular and cerebellar stimulation are marked by solid vertical bars.

The data plotted in Figure 17 show that presynaptic inhibition of the quadriceps potentials is completely prevented by a cummulative dose of 0.75mg/kg of bicuculline given over a 15 min interval, but direct inhibition is completely unaltered. Presynaptic inhibition of the biceps-semitendinosus potentials elicited by conditioning stimulation of the quadriceps nerve

showed a mean percent change of $36.5 \pm 12.7\%$ (n= 8, P< .025), $59.1 \pm$
14.9% (n= 9, P< .005) and $72.6 \pm 13.3\%$ (n= 9, P< .005) with cummulative
doses of 0.25 mg/kg, 0.5 mg/kg, and 1.0 mg/kg, respectively, of
bicuculline. Although the early phase of presynaptic inhibition (5-30 msec.)
could usually be completely blocked by bicuculline, it was observed in
four experiments that a much later phase of presynaptic inhibition (40-
100 msec.) was unaffected or actually potentiated by bicuculline. In all
experiments in which direct inhibition of the segmental potentials was
studied, bicuculline had little or no effect on this inhibition in doses as
large as 1.0 mg/kg.

In six experiments, a cummulative dose of 0.5 mg/kg produced a mean
percent reduction in cerebellar inhibition of the quadriceps potentials of
$47.4 \pm 23.4\%$ (P< 0.05). This is illustrated in Figure 1 by the solid vertical
bars labelled C_1 and C_2. Cerebellar inhibition of the biceps semitendinosus
potentials showed a mean percent reduction in inhibition of $5.5 \pm 14.0\%$
(n=11), $61.3 \pm 11.8\%$ (n=13, P< .005) and $73.0 \pm 12.5\%$ (n=12, P< .005)
with cummulative doses of 0.25 mg/kg, 0.5 mg/kg and 1.0 mg/kg, respec-
tively of bicuculline. In about one half of these experiments it was observed
that small doses of bicuculline (0.25 mg/kg), initially potentiated the
cerebellar inhibition instead of suppressing it. An example of this
potentiation after an intravenous injection of 0.25 mg/kg of bicuculline is
shown in insert B in Figure 17. With an additional 0.5 mg/kg of bicuculline,
this potentiating eff   completely reversed and all cerebellar inhibition
of the quadriceps p   was abolished. Cerebellar inhibition of the
segmental monosynaptic action potentials was unaffected by doses of

bicuculline as large as 1.0 mg/kg in only three out of nineteen experiments.

Reticular inhibition of the segmental potentials was unaffected or only slightly reduced by accumulative doses of bicuculline up to 1.0 mg/kg. The vertical bars marked R_1 and R_2 illustrate the lack of effect of 0.5 mg/kg of bicuculline on reticular inhibition of the quadriceps potentials as determined by five experiments.

These present experiments support the findings of Curtis, Duggan, Felix and Johnston, 1970, that bicuculline has no effect on direct inhibition and extend these findings to include reticular inhibition in this category. Thus it seems very unlikely that GABA plays any role in the mediation of reticular inhibition of the alpha motoneurons.

The blockade of cerebellar disfacilitation by bicuculline was expected since its proposed mechanism is Purkinje cell inhibition of lateral vestibular neurons (Llinas, 1964) and Curtis, Duggan and Felix, 1970 have shown that bicuculline blocks this inhibition. Also, earlier studies with diphenylamino-ethanol showed that this compound blocked cerebellar disfacilitation of the segmental monosynaptic action potentials (Huffman and Yim, 1969) and it too, like bicuculline, blocks GABA induced inhibition of lateral vestibular neurons (Kee, Wells and Yim, 1971).

Although it has been reported that bicuculline does not block presynaptic inhibition of the gastrocnemius motoneurons (Curtis, Duggan, Felix and Johnston, 1970), we have found that the early phase (5-30 msec.) of presynaptic inhibition of either the quadriceps or biceps semitendinosus monosynaptic action potentials is consistently blocked by bicuculline. These findings are supported by the observations of Levy _et al._ that

primary afferent depolarization which is presumably associated with

presynaptic inhibition is also blocked by bicuculline.

2.   Work to be performed

We are just beginning our investigation of the effects of intravenously

administered psychotomimetic drugs on Purkinje and Deiter's neuronal

activity.   Our initial observation of increased neuronal discharge by

Deiter's neurons after intravenous LSD are encouraging.   I plan to continue

my investigations of the effects of LSD and the neuronal discharge patterns

of Deiter's neurons and extend this study to include other psychotomimetic

drugs such as mescaline, bufotenine and the amphetamines.   Similar

studies will be conducted on Purkinje neuronal discharge patterns.

To determine if these drugs are mimicing the action of noradranline

on Purkinje cells, i.e., inhibiting Purkinje cell discharge, I plan to

study the effect of the psychotomimetic drugs on Purkinje cell discharge

when these drugs are applied microiontophoretically through glass micro-

pipettes to single Purkinje cells. An inhibition of Purkinje cell discharge

would reduce the inhibitory input to Deiter's neurons and explain their

increased discharge rate after LSD and also explain the suppression of

cerebellar disfacilitation.

3.   Publications

1.   Huffman, Ronald D., The effect of bufotenine on cerebellar

disfacilitation in cats. XXV International Physiological Congress, Munich,

Germany, July 25-31, 1971 (Abstract to be published in The Proceeding

of the XXV International Physiological Congress).

2.   Huffman, Ronald D., Bicuculline blockade of cerebellar

disfacilitation and presynaptic inhibition. Fall Pharmacology Meetings,

Burlington, Vermont, August 22-26, 1971. (Abstract to be published

in The Pharmacologist).

3.   Huffman, R. D. and McFadin, L., Supression of presynaptic

inhibition and cerebellar disfacilitation by bicuculline. Submitted to Brain

Research, June, 1971.

4.   Huffman, R. D. and McFadin, L. The effects of bicuculline on

spinal and supraspinal inhibition in cats. In preparation and to be submitted

to Int. J. Neuropharmacology.

4.   References

1.   Curtis, D. R., Duggan, A. W. and Felix, D., GABA and

inhibition of Deiters' neurones, Brain Research, 23 (1970) 117-120.

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4.   Huffman, R. D. an Yim, G. K. W., Effects of diphenylamino-

ethanol and lidocaine on central inhibition, Int. J. Neuropharmac., 8

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5.   Kee, R. D., Wells, J. N. and Yim, G. K. W., Antagonism by

diphenylaminoethanol of GABA inhibition of Deiters neurons, Fed. Proc.,

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6.   Levy, R. A., Repkin, A. and Anderson, E. G., Bicuculline

blockade of primary afferent depolarization, Fed. Proc., 30 (1971)

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7.   Llinas, R., Mechanisms of supraspinal actions upon spinal

cord activities. Differences between reticular and cerebellar inhibitory

actions upon alpha extensor motoneurons, J. Neurophysiol., 27 (1964)

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properties of the postsynaptic inhibition by Purkinje cell axons and the

action of γ-aminobutyric acid on Deiters neurones, Exp. Brain Res.,

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9.   Obata, K., Otsuka, M. and Tanaka, Y., Determination of

gamma-aminobutyric acid in single nerve cells of cat central nervous

system, J. Neurochem., 17 (1970) 697-698.

Legends of Figures:

Figure 1.   Effects of LSD on cerebellar disfacilitation of extensor mono-

synaptic action potentials. Decerebrate cat, immobilized with gallamine.

A. Cerebellar disfacilitation of the quadriceps monosynaptic action

potentials (8V, 200/sec, 0.1 msec for 9 sec, bipolar silver ball electrodes).

Line under responses indicates duration of inhibitory stimulus. B. 1 min.

after 100µg/kg LSD. C. Same response but 1 min after 200µg/kg LSD

given intravenously as an accumulated dose over an 8 min period. D.

1 hour la r.

Figure 2.   Effects of LSD on cerebellar disfacilitation of flexor mono-

synaptic action potentials. Decerebrate cat. A.. Cerebellar

disfacilitation of the biceps semitendinosus monosynaptic action potentials

(6V, 200/sec, 0.4 msec for 9 sec, bipolar silver ball electrodes). B.

3 min after 50µg/kg LSD given intravenously. C. 11 min after 100µg/kg

LSD given intravenously as an accumulated dose over a 4 min period.

D. 50 min later.

Figure 3.   Effects of LSD on cerebellar disfacilitation of flexor mono-

synaptic action potentials of the segmental reflex. Decerebrate cat,

immobilized with gallamine. A. Cerebellar disfacilitation of the

biceps semitendinosus monosynaptic action potentials (40V, 150/sec,

1 msec for 9 sec, bipolar silver ball electrodes). Line under responses

indicates duration of inhibitory stimulus. B. 3 min after 30µg/kg LSD

given intravenously. C. Same responses but 3 min after 300µg/kg LSD

given intravenously as an accumulated dose over a 24 min period.

E. 40 min later.

Figure 4.   Effects of bufotenine on cerebellar disfacilitation of extensor

monosynaptic action potentials. Decerebrate cat, immobilized with

gallamine. A. Cerebellar disfacilitation of the quadriceps mono-

synaptic action potentials (15V, 200/sec, 0.1 msec for 15 sec, bipolar

silver ball electrodes). B. 2 min after 0.5 mg/kg bufotenine given

intravenously.

Figure 5.   Effects of bufotenine on cerebellar disfacilitation of extensor
monosynaptic action potentials. Decerebrate cat, immobilized with
gallamine. A. Cerebellar disfacilitation of the quadriceps monosynaptic
action potentials (8V, 200/sec, 0.25msec for 9 sec, bipolar silver ball
electrodes). B. 5 min after 0.25 mg/kg bufotenine given intravenously.
C. 8 min after 0.5 mg/kg bufotenine given intravenously as an accumulated
dose over a 10 min period. D. 1 hour later.

Figure 6.   Effects of mescaline on reticular inhibition of flexor mono-
synaptic action potentials. Barbiturate anesthetized (Nembutal) cat.
A. Reticular inhibition of the biceps semitendinosus monosynaptic
action potentials (5V, 100/zec, 0.6 msec for 5-10 sec, bipolar concentric
electrode). B. 2 min after 10mg/kg mescaline given intravenously over
a 10 min period.

Figure 7.   The effect of Δ^1-tetrahydrocannabinol (THC) on reticular
inhibition of extensor monosynaptic action potentials. Decerebrate cat,
immobilized with gallamine. A. Reticular inhibition (2V, 100/sec,
0.6 msec for 10 sec, bipolar concentric electrode). Line under responses
indicates duration of inhibitory stimulus. B. Same responses but 2 min after
300µg/kg THC given intravenously as an accumulated dose over a 6 min
period. C. 10 min later. D. 1 hour later.

Figure 8.   Effects of psilocybin on reticular inhibiton of the patellar and linguomandibular reflexes. Cat anesthetized with chloraloseurethane. A. and B. Reticular inhibition (5V, 100/sec, 0.6 msec for 6 sec, bipolar concentric electrodes) of the monosynaptic patellar reflex and multisynaptic linguomandibular reflex, respectively, before and after 10µg/kg psilocybin. C. Duration of reticular stimulation. D. Blood pressure record.

Figure 9.   Effects of LSD on reticular inhibition of the monosynaptic patellar reflex. Chiorale. e-urethane anesthetized cat. A. Reticular inhibition (3.6V, 100/sec, 0.6 msec for 15 sec, bipolar concentric electrode) of the monosynaptic patellar reflex before and after 60µg/kg LSD given intravenously. Blood pressure response is illustrated in the lower part of each record. The record is continuous from A through C. B. Same responses but after an additional 100µg/kg LSD. C. Same responses but after an additional 100µg/kg LSD (total accumulated dose of 260µg/kg given intravenously over a 22 min period).

Figure 10.   Effects of bufotenine on reticular inhibition of the monosynaptic patellar reflex. Chloralose-urethane anesthetized cat. A. and B. Reticular inhibition (2V, 100/sec, 0.6 msec for 15 sec, bipolar concentric electrode) before and after 1 mg/kg bufotenine given intravenously as an accumulated dose over a 20 min period. Blood pressure response is illustrated in the lower part of each record. C. 1 hour after the last dose of bufotenine. (The changes in blood pressure observed during reticular stimulation are a result of stimulation of the medullary pressor and depressor centers).

Figure 11.   Effects of Ditran on reticular inhibition of the monosynaptic

patellar reflex. Chloralose-urethane anesthetized cat. A. Reticular

inhibition (3V, 100/sec, 0.6 msec for 10 sec, bipolar concentric

electrode) of the monosynaptic patellar reflex. Blood pressure response

is illustrated in the lower part of each record. The record from A

through D is not continuous. B. Same responses but after 7mg/kg

Ditran given intravenously as an accumulated dose over a 5 min period.

C. Same responses but after 15mg/kg Ditran given intravenously as an

accumulated dose over a 10 min period.

Figure 12.   Effects of Ditran on reticular inhibition of the monosynaptic

patellar reflex. Chloralose-urethane anesthetized cat. A. Reticular

inhibition (5.2 - 5.6V, 100/sec, 0.6 msec for 10 sec, bipolar concentric

electrode) of the monosynaptic patellar reflex. The record from A

through C is not continuous. B. Same responses but after 6 mg/kg

Ditran given intravenously as an accumulated dose over a 4 min period.

C. Same responses but 1 min after 12mg/kg Ditran given intravenously

as an accumulated dose over a 10 min period.

Figure 13.   Effects of bicuculline on cerebellar disfacilitation and pre-

synaptic inhibition of extensor monosynaptic action potentials. Decere-

brate cats, immobilized with gallamine. A. Control record showing

cerebellar disfacilitation (4V, 200/sec., 0.4 msec. for 9 sec., bipolar

silver ball electrodes) and presynaptic inhibition (0.9V conditioning

pulse applied to the antagonistic biceps-semi-tendinosus nerve, 10

msec. delay) of the quadriceps monosynaptic action potentials. Line

under responses indicates the duration of the inhibitory stimulation.
B. Same responses 11 min after 0.25mg/kg bicuculline given intra-
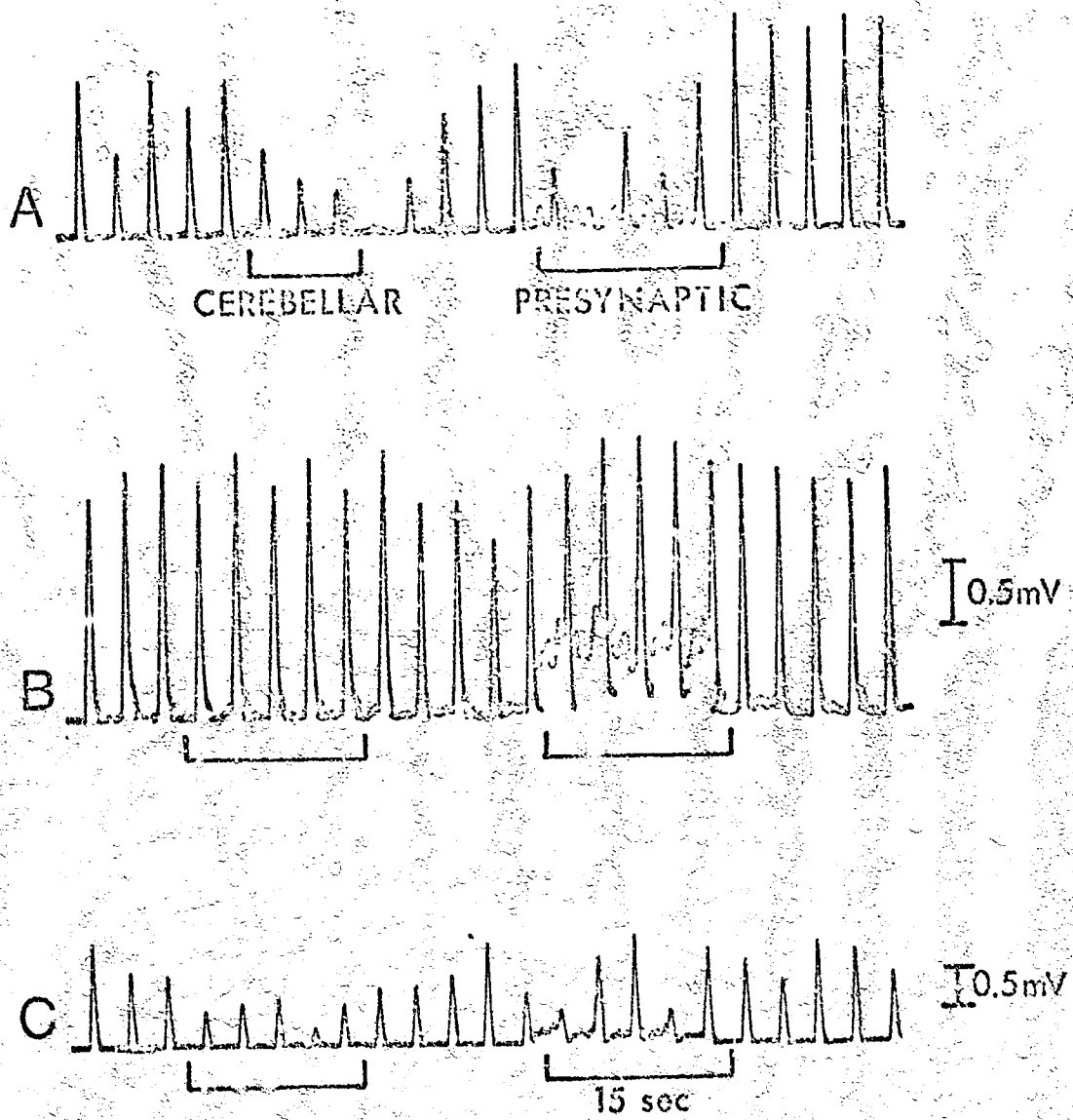venously. C. Same responses 45 min after the above injection of
bicuculline.

Figure 14. Effects of bicuculline on presynaptic inhibition and cerebellar
disfacilitation of extensor monosynaptic action potentials. Decerebrate
cats, immobilized with gallamine. A. Control record showing
presynaptic inhibition (1.2V conditioning pulse, 0.2 msec. duration
applied to the antagonistic biceps-semitendinosus nerve, 10 msec
delay) and cerebellar disfacilitation (2V, 100/sec., 0.4 msec for 9 sec.,
bipolar concentric electrode) of the quadriceps monosynaptic action potentials.
Line under responses indicates the duration of the inhibitory stimulation.
B. Same respon.es but 7 min after 0.25mg/kg bicuculline given intra-
venously. C. 4 min. after 0.5mg/kg bicuculline given intravenously
as an accumulated dose over a 15 min interval. D. 20 min later.
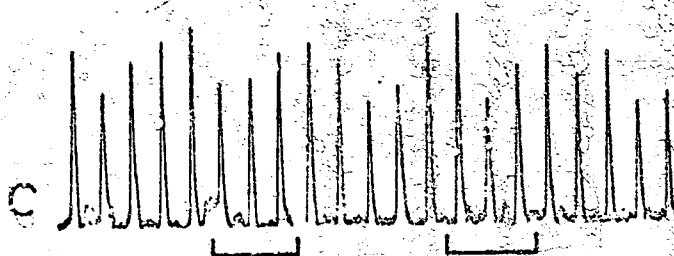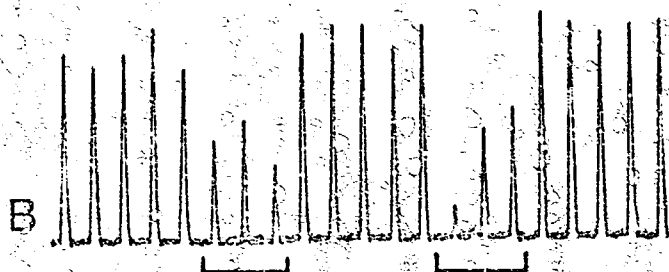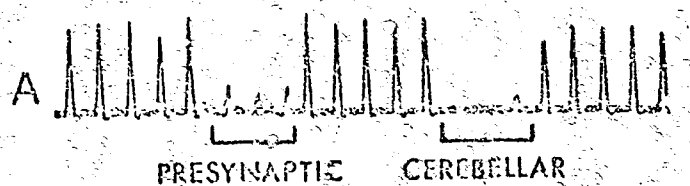
Figure 15. Effect of bicuculline on direct and presynaptic inhibition of flexor
monosynaptic action potentials. Decerebrate cat, immobilized with gallamine.
A. Control record showing direct and presynaptic inhibition of the biceps-
semitendinosus potentials induced by a conditioning stimulation (1.2V, 0.2
msec) in the antagonistic quadriceps nerve with 1 msec and 10 msec delays,
respectively. Line under responses indicates the duration of the inhibitory
stimulus. B. Same responses 6 min after 0.25mg/kg bicuculline given
intravenously. C. 4 min after 0.75mg/kg bicuculline given intravenously
as a cumulative dose over a 15 min interval. D. 15 min after 0.75mg/kg
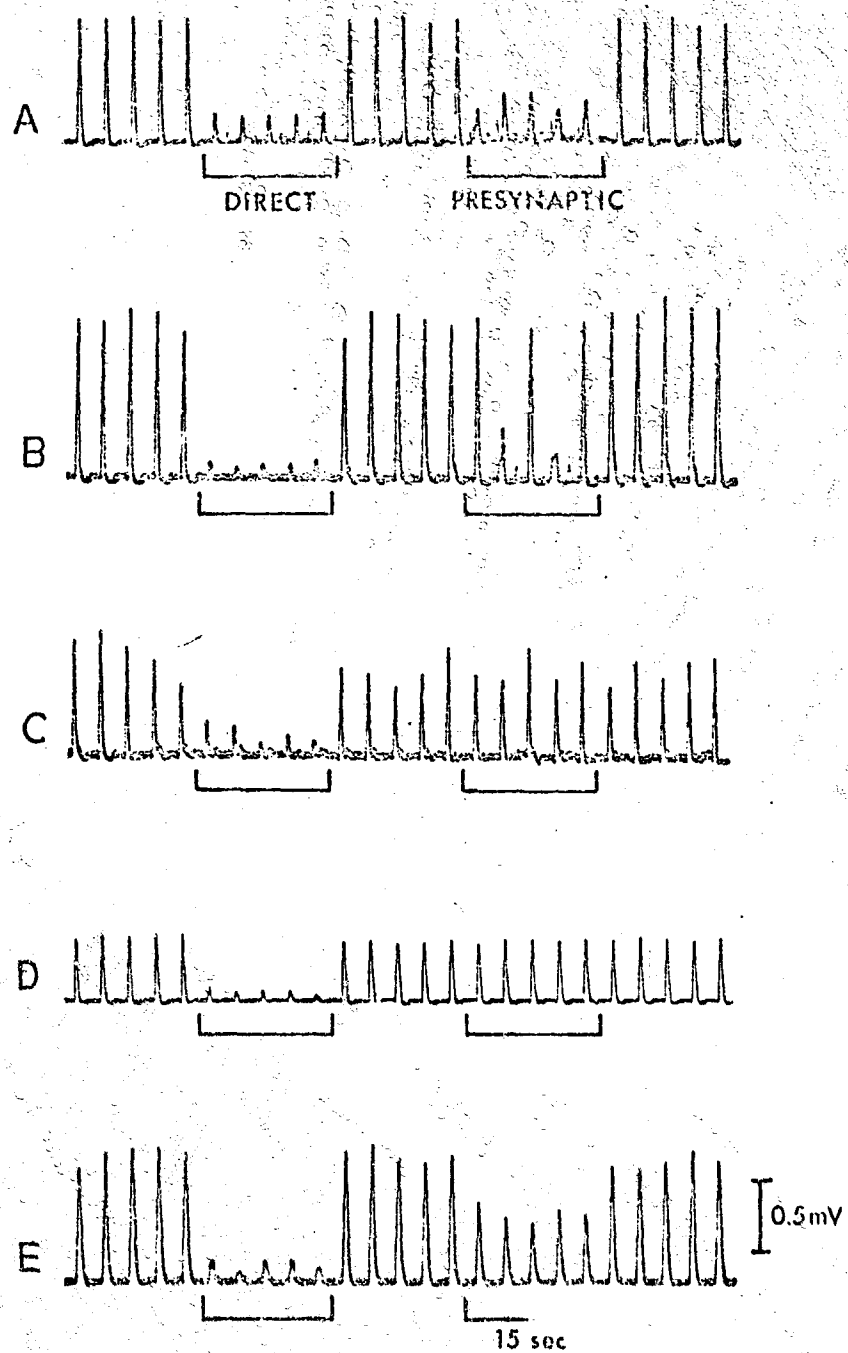bicuculline. E. 1 hour later.

Figure 16.   Effects of bicuculline on direct, presynaptic and reticular

inhibition of flexor monosynaptic action potentials. Decerebrate cat,

immobilized with gallamine. A.   Control record showing direct and

presynaptic inhibition of the biceps-semitendinosus potentials by a

conditioning stimulus (0.1V, 0.2 msec and 1.0V, 0.2 msec,

respectively) in the antagonistic quadriceps nerve with 1 msec and

25 msec delays, respectively.   Line under responses indicates

duration of inhibitory stimulation.   B.   5 min after 0.5mg/kg bicuculline

given intravenously as an accumulative dose over a 7 min interval.

C.   Control record showing presynaptic (1V, 0.1 msec, 20 msec delay)

and reticular (0.6V, 100/sec for 15 sec, bipolar concentric electrode)

inhibition of the biceps-semitendinosus potentials.   D.   Same responses

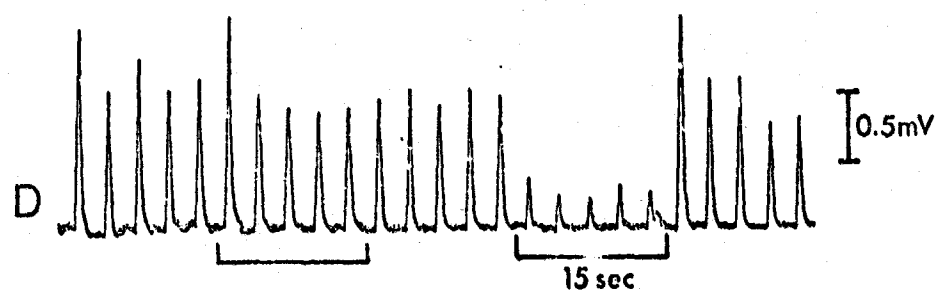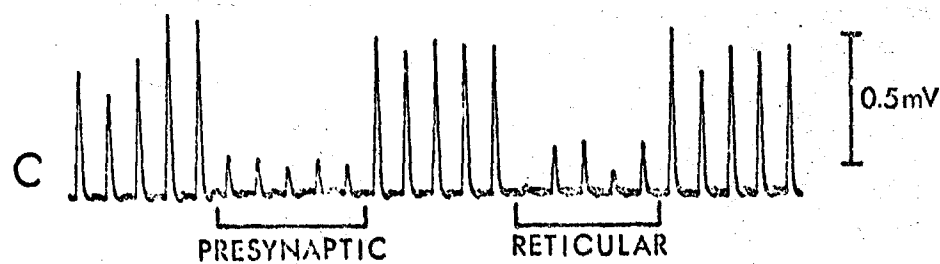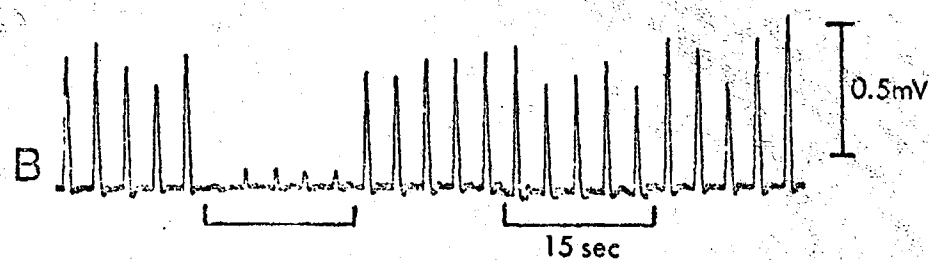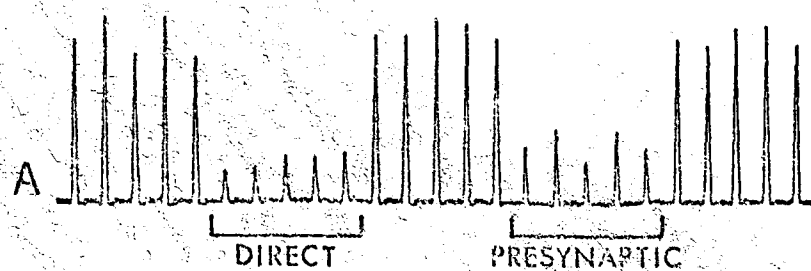but 12 min after 0.25mg/kg bicuculline given intravenously.

Figure 17.   Effects of bicuculline upon direct, presynaptic and reticular

inhibition and cerebellar disfacilitation of the extensor segmental

monosynaptic action potentials.   Ordinate: percent height of the

quadriceps monosynaptic action potentials (ventral root recording) at

different times (abscissa) following a single shock stimulation of the

antagonistic biceps-semitendinosus nerve.   The solid curve represents

the control excitability curve of a single experiment.   The broken curve

represents the excitability curve recorded 15 min after 0.75mg/kg of

bicuculline given I.V. as an accumulate dose over a 15 min interval.

The superimposed solid vertical bars R_1 and C_1 represent percent

height of the quadriceps potentials following stimulation of the reticular

formation (n=5) and the cerebellum (n=6), respectively. Solid vertical

bars R_2 and C_2 represent these same responses 10 min after 0.5mg/kg

bicuculline given I.V. Insert figure A is a control record illustrating

cerebellar disfacilitation (8V, 200c/s, 0.4 msec for > sec, bipolar

silver-ball electrodes) of the quadriceps potentials. Line under responses

indicates duration of inhibitory stimulation. B. Same responses but 13

min after 0.25mg/kg bicuculline given I.V. C. 15 min after 0.75mg/kg

bicuculline given I.V. as an accumulate dose over a 15 min interval.

D. Same responses 45 min after the above dose of bicuculline.

FIG 13.   299.

CEREBELLAR   PRESYNAPTIC

15 sec

FIG. 14

FIG.15

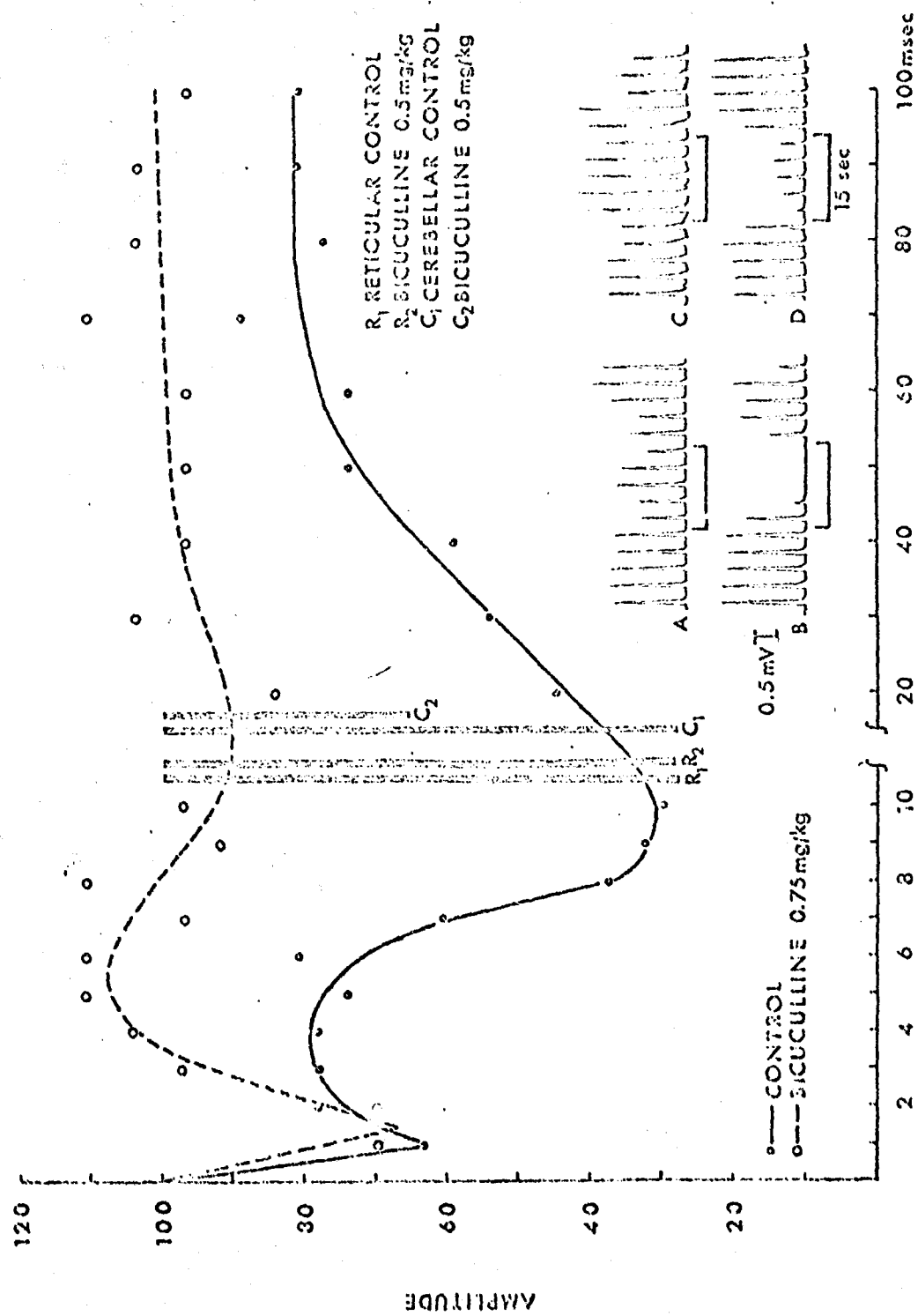A — DIRECT — PRESYNAPTIC

0.5 mV

15 sec
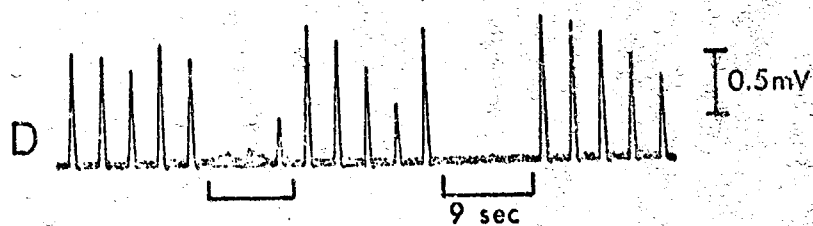
TABLE 1. Effects of bicuculline on cerebellar disfacilitation and reticular inhibition of the monosynaptic segmental potentials in decerebrate cats immobilized with gallamine

Cumulative Dose (i.v.)	Source of Inhibition	Reflex Inhibited	Mean Percent Change in inhibition ± S. E.	P Values[*]	n (no. of experiments)
0.25 mg/kg	Cerebellar	Flexor	5.5 ± 14.0		11
		Extensor	21.3 ± 27.4		6
	Reticular	Flexor	9.7 ± 29.6		4
		Extensor	7.2 ± 23.3		5
0.5 mg/kg	Cerebellar	Flexor	61.3 ± 11.8	P< .005	13
		Extensor	47.4 ± 23.4	P< .05	6
	Reticular	Flexor			
		Extensor	5.8 ± 26.4		5
1.0 mg/kg	Cerebellar	Flexor	73.0 ± 12.5	P<.005	12
		Extensor	75.0 ±		4
	Reticular	Flexor			
		Extensor	-21.6 ± 1.7		2

*A one-tailed Student's "t" test was performed on each mean percent change in inhibition

TABLE 2. Effects of bicuculline on direct and presynaptic inhibition of the monosynaptic segmental potentials in decerebrate cats immobilized with gallamine

Cumulative Dose (i.v.)	Source of Inhibition	Reflex Inhibited	Mean Percent Change in inhibition ± S.E.	P Values*	n (no. of experiments)
0.25 mg/kg	Direct	Flexor	6.6 ± 16.2		8
		Extensor	-13.2 ± 17.0		8
	Presynaptic	Flexor	36.5 ± 12.7	P< .025	8
		Extensor	35.9 ± 13.4	P< .025	6
0.5 mg/kg	Direct	Flexor	17.5 ± 19.7		8
		Extensor	5.9 ± 19.6		5
	Presynaptic	Flexor	59.1 ± 14.9	P< .005	9
		Extensor	78.4 ± 11.6	P< .005	6
1.0 mg/kg	Direct	Flexor			
		Extensor			
	Presynaptic	Flexor	72.6 ± 13.3	P< .005	9
		Extensor	88.3 ±		5

*A one-tailed Student's "t" test was performed on each mean percent change in inhibition

THE EFFECT OF BUFOTENINE ON CEREBELLAR DISFACILITATION IN CATS.
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Intravenous injection of bufotenine (5.0 mg/kg) in monkeys has been reported to produce a prone position in which these animals cannot walk or climb. This is followed by a period of marked ataxia and heightened tendon reflexes. In humans, nystagmus has been reported. These observations suggest that bufotenine may be acting at the level of the cerebellum to alter normal cerebellar somatomotor control. Since the entire outflow from the cerebellar cortex (Purkinje/neurons) is inhibitory in function and since some of the functional observations suggest a reduction of inhibitory input to spinal motoneuron this study was designed to investigate the effect of bufotenine on cerebellar inhibition (disfacilitation) of the segmental (quadriceps and biceps-semitendinosus) monosynaptic action potentials. Midcollicular decerebrate cats were used in these experiments. Surface stimulation of the vermis of the anterior lobe of the cerebellum with bipolar silver ball electrodes was used to induce the disfacilitation of the segmental potentials. Intravenous injections of bufotenine (0.25-).5 mg/kg) markedly reduced or completely eliminated the cerebellar disfacilitation of both segmental potentials; the depression of this disfacilitation lasted approximately 50 min. The segmental potentials were unaffected or slightly reduced by these same doses; this effect, however, was quite variable. Direct inhibition of the biceps monosynaptic potentials induced by stimulation of the antagonistic quadriceps nerve was unaffected by bufotenine (2.0 mg/kg). Reticular inhibition was unaffected or only slightly reduced with doses of bufotenine as large as 1.5 mg/kg. (Supported by Morrison Trust (R-A-16) and AF-70-C-0059).

BICUCULLINE BLOCKADE OF CEREBELLAR DISFACILITATION AND PRE-
SYNAPTIC INHIBITION. Ronald D. Huffman* (SPON: A.R. Briggs).
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Recent experimental studies have suggested that gamma-amino
butyric acid (GABA) might be the inhibitory transmitter re-
leased by Purkinje cells of the cerebellum and by spinal
neurons involved in presynaptic inhibition. To further test
this hypothesis, the effects of the GABA antagonist bicucul-
line were tested on spinal (direct and presynaptic) and supra-
spinal (reticular and cerebellar) inhibition of the segmental
monosynaptic action potentials. Midcollicular decerebrate
cats paralyzed with Flaxedil were used in these experiments.
Bicuculline (0.25-0.5 mg/kg i.v.) markedly reduced or com-
pletely eliminated the cerebellar disfacilitation and the
early (10-20 msec.) presynaptic inhibition of the segmental
potentials. Reticular and direct (1-2 msec.) inhibition were
unaffected by these doses. Larger doses of bicuculline (1 mg/
kg) produced only slight reduction in reticular inhibition of
these potentials. The duration of action of bicuculline was
short (20-25 min.) and the inhibitory phenomena returned to
predrug levels within this time. These studies are in agree-
ment with the suggestion that GABA may be the transmitter sub-
stance involved in spinal presynaptic inhibition and Purkinje
mediated cerebellar disfacilitation. (Supported by Morrison
Trust (R-A-16) and AF-70-C-0059).

Central Nervous System

A. Study of the effects of acetylcholine and cholinesterase inhibitors on the central nervous system.

   1. Progress Report

      a. Acetylcholinesterase: increase in the hippocampus during learning.

         The cholinesterase inhibitor disopropylflurophosphate when given to rats causes a decrease in extinction, a decrease in efficiency in serial problem solving and produces amnesia. However, reported changes of cholinesterase activity as it relates to learning have been limited to: (1) The effect of long term environmental stimuli in rats, which causes a slight increase in the brain's activity and, (2) The finding that the cholinesterase of the metathoracic ganglion of cockroaches decreases with training. The use of ablation methods have indicated the involvement of the hippocampus in the process of learning. Therefore, cholinesterase activity in the hippocampus and caudate nucleus was studied immediately after a learning task. The cage was divided by a partition so that the trained animal and the control-shocked animal were on the same grid and received the same shock. The other control remained in his cage. The rats were trained for $3\frac{1}{2}$ days, two 30 minute sessions per day. During this period they learned to avoid the shock. Immediately after the last training period, they were sacrificed using a guillotine. The caudate nucleus and the hippocampus were dissected out, cooled with ice and then assayed for acetylcholinesterase and butyrylcholinesterase activity

by the method of Siakotos. Protein was estimated using the method
of Lowry. Cholinesterase was calculated both in activity per weight
of tissue and per gram of protein. Since there was essentially
no difference between the two, only the cholinesterase activity
per gram of protein is reported. Three separate groups of 24 rats
were trained. In each case, the cholinesterase in the hippocampus
in the trained rats was significantly higher than that of the
control or control-shocked rats. The butyrylcholinesterase activity
of the hippocampus was not significantly different in any of the
rats. Both the acetylcholinesterase and butyrylcholinesterase
activity in the caudate nucleus were not significantly different.
Another group of rats was trained in the same way and the hippocampus
was divided into two areas, the regio superior and the area dentata.
The major difference in cholinesterase activity seems to occur in
the regio superior. Another group of rats were then trained in
the same way and killed one week late. and the cholinesterase
measured. The cholinesterase activity in the hippocampus was no
longer significantly different from that of the control animals.
The change in activity of cholinesterase is not a permanently
induced change. Acetylcholinesterase in the hippocampus is involved
in the learning process. The change of acetylcholinesterase in the
hippocampus could be occurring in response to increased acetylcholine
release or could be occurring as a control mechanism to modulate the
amount of acetylcholine available at the receptor. These changes

could be occurring to establish neuronal pathways during the
learning process and after the pathway has been established or
utilized, the cholinesterase returns to control levels.

b. Effect of ethanol on the cholinesterase activity in the brain of
the rat.

A number of recent studies indicate that ethanol inhibits the
release and increases the content of acetylcholine in the brain.
It also reduced the cholinesterase, CoASH and choline acetylase
activity in the supernate isolated from the brain homogenate. This
study was undertaken to investigate the effects of ethanol, on
acute and chronic administration, on the cholinesterases in discrete
parts of the brain. Texas inbred male albino rats, weighing 200-
300 gm were used. Rats received, P.O., either acutely or for 7 days,
6.83 gm/kg, of 44% ethanol. Others received 22% ethanol in their
drinking water for 6 weeks. Appropriate controls were used. Rats
were sacrificed by decapitation. The cholinesterase activity in
the medulla, hypothalamus, caudate, hippocampus, anterior thalamic
nuclei and liver were estimated employing labeled acetylcholine
and butyrylcholine. These data indicate that ethanol has no signi-
ficant effect on the true and pseudo-cholinesterase activity in
the discrete parts of the brain of the rat.

c. Effects of chronic disulfoton treatment on the cholinesterase
activity of the rat.

The purpose of this investigation was to study the effect of

chronic disulfoton treatment on acetylcholinesterase activity (AchE) and butyrylcholinesterase activity (BUchE). Thirty Holtzman rats weighing 100-130 gm were divided into three groups of 10. Two groups recieved 1.5 mg/kg, I.P. of disulfoton for 10 days; the third group received only the vehicle. One of the two treated groups was allowed to recover for 7 days. Rats were sacrificed by decapitation. The AchE and BUchE activities in mMoles/g protein/ hr. were estimated in Hypothalamus (Hy), Medulla (M), Hippocampus (Hi), Caudate Nucleus (CN), Ileum (I) and Gastrocnemius (G) employing labelled acetylcholine (Ach) and butyrylcholine as substrates. Control AchE activities were: M 5.2599, Hy 4.6205, Hi 3.1906 and CN 13.5607, activities in the treated group were: M 1.9546, Hy 1.8724, Hi 0.6245 and CN 2.4229 and activities in the recovery group were: M 4.0020, Hy 3.2781, Hi 1.7921 and CN 8.6547. CN had the highest activity of the components studied while Hi had the lease AchE activity. Disulfoton inhibited the AchE activity in the CN 83%, Hi 81%, M 63%, and Hy 60%. The AchE activity in the ileum was: control 2.9041, poisoned 2.3775 and recovery 2.8064. The gastrocnemius AchE activity was: control 0.4817, poisoned 0.2774, recovery 0.4022. Disulfoton had little or no effect on ileum AchE. However, it inhibited AchE to 43% in gastrocnemius muscle.

d. Microwave radiation to inactivate cholinesterase in the rat brain prior to analysis for acetylcholine.

Since rapid inactivation of enzyme activity is a necessity

for the accurate estimation of acetylcholine concentration and turn-
over in the brain, inactivation by rapid heating with 2450 MHZ
microwave radiation was studied. The results were:

ACETYLCHOLINE µg/g ± SD

Sample Preparation	Time Sec.	Assay Method	Control	Cholinesterase Inhibited
Microwave	11	g.p. ileum	4.05±1.18 (13)	6.09±1.15 (9)
Decap. & Freeze[a]	30+	g.p. ileum	2.65±0.11 (4)	4.54±0.10 (4)
Microwave	11	g. chromat.	4.82±0.57 (7)	5.43±1.12 (9)
Decap. & Freeze[a]	30+	g. chromat.	3.81±0.09 (4)	5.44±0.20 (4)

[a]J. Pharmacol. Exp. Therap. 150: 231, 1965.

These results indicate that the use of microwave radiation decreases

the time required for enzyme inactivation and increases the

concentration of acetylcholine measured.

2. Work To Be Performed

a. Studies will be continued on the cholinergic component in

learning to further localize the changes within the brain. We

will also study the changes that occur in acetylcholine concentration

and turnover.

b. Further studies will be carried out on the effects of cholinesterase

inhibition on the functions of the brain and animal performance.

c. The use of microwaves to inactivate enzymes for analysis of concen-

tration and turnover will continue.

PUBLICATIONS:

Microwave radiation to inactivate cholinesterase in the rat brain prior to
analysis for acetylcholine. William B. Stavinoha, Barbara Pepelko and
Paul W. Smith, Pharmacologist, 12:257, 1970.